THE UNIVERSITY OF LIVERPOOL

ANNALS
OF
TROPICAL MEDICINE AND PARASITOLOGY

ISSUED BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

Edited by

PROFESSOR WARRINGTON YORKE, M.D., F.R.C.P., F.R.S.
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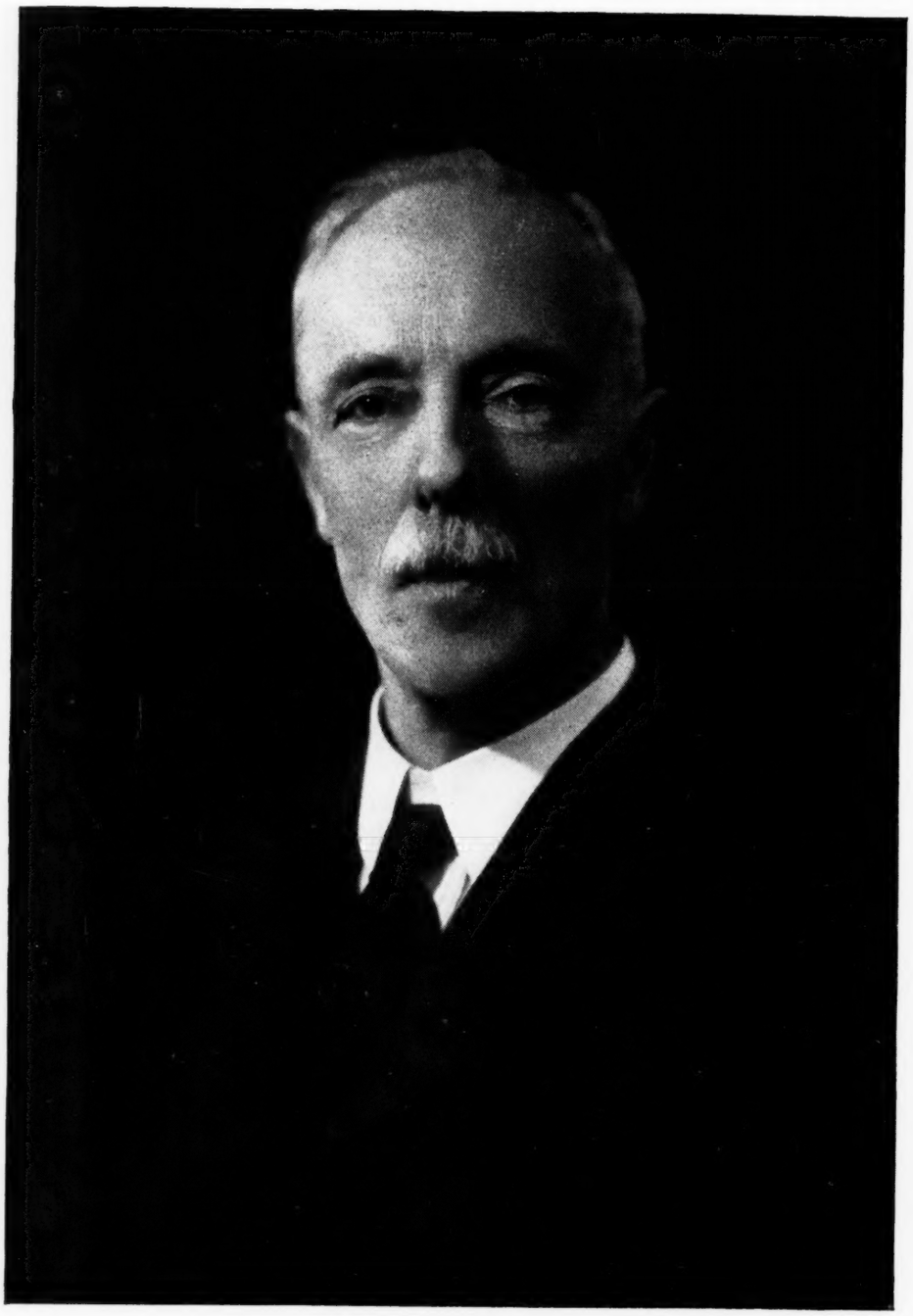
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Arthur S. Bayliss

Volume 30 April 8th, 1936 No. 1

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(2) Two courses for the Diploma in Tropical Hygiene, commencing on the 23rd April, 1936, and the 7th January, 1937. The D.T.H. examinations are held in March and June.

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1914	Arculli, Hassan el
1914	Chohan, Noormahomed Kasembha
1914	Connell, Harry Bertram
1914	Gerrard, Herbert Shaw
1914	Gimi, Hirji Dorabji
1914	Gwynne, Joseph Robert
1914	Hodkinson, Samuel Paterson
1914	Jackson, Arthur Ivan
1914	Kaushash, Ram Chander
1914	Kelsall, Charles
1914	Luanco y Cuenca, Maximino
1914	Misbah, Abdul-Ghani Naguib
1914	Naidu, Bangalore Pasupulati Balakrishna
1914	Rowe, John Joseph Stephen
1914	Roy, Raghu Nath
1914	Shiveshwarkar, Ramchandra Vishnu
1914	Sur, Sachindra Nath
1914	Talati, Dadabhai Cursedji
1914	Wilkinson, Arthur Geden
1914	Wright, Ernest Jenner
1915	Lobo, John Francis
1915	Madhok, Gopal Dass
1915	Pearson, George Howorth
1915	Swami, Karumuri Virabhadra
1915	Wood, John

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1916	Barseghian, Mesroob
1916	Chaliha, Lakshmi Prasad
1916	Lim, Albert Liat Juay
1916	Lim, Harold Liat Hin
1916	Metzger, George Nathanie
1916	Söderström, Erik Daniel
1916	Wheeler, Louis
1917	Chapman, Herbert Owen
1917	Krishnamoorthy, Yedatore Venkoba
1917	Lipkin, Isaac Jacob
1918	Watts, Rattan Claud
1919	Bowle-Evans, Charles Harford
1919	Burnie, Robert McColl
1919	Celestin, Louis Abel
1919	Cummings, Eustace Henry Taylor
1919	Darling, Georgina Renington
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1919	Fraser, William James
1919	Gordon, Rupert Montgomery
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1919	Oluwole, Isaac Ladipo
1919	Rustomjee, Khusshuyee Jamesidjee
1919	Sawers, William Campbell
1919	Thompson, Mary Georgina
1919	Turner, Gladys Maude
1919	Young, Charles James
1920	Adler, Saul
1920	Anderson, William Jenkins Webb
1920	Campbell, George
1920	Cobb, Charles Eric
1920	Cobb, Enid Margaret Mary
1920	Connolly, Evelyn Mary
1920	Fernandez, Daniel David
1920	Lim, Chong Eang
1920	McHutchison, George Browne
1920	van der Merwe, Frederick
1920	O'Farrell, Patrick Theodore Joseph
1920	Renner, Edowo Awunor
1920	Vaughan, James Churchwill
1920	Waller, Harold William Leslie
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1921	Corfield, Charles Russell
1921	Hamid, Abdul
1921	Longhurst, Bell Wilmott
1921	Macvae, George Anthony
1921	Madan, Hans Raj
1921	Mulligan, William Percival
1921	Nixon, Robert
1921	Richmond, Arthur Stanley
1921	Shri Kent, Shamsher Singh
1921	Skinner, James Macgregor
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1922	Cohen, Morris Joshua
1922	Crawford, Andrew Clemmey
1922	Gilmore, Edward Raymond
1922	Gracias, Cajetan Manuel
1922	Jennings, Arthur Richard
1922	Lethem, William Ashley
1922	Paul, Sachchidananda Hoshen
1922	Pinder, John
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1923	Abelman, B.
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1923	Edghill, Winifred M.
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1925	Mackay, E. K.
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1927	Barrowman, B.

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1928	Krishna, R.
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1928	Lawrence, M. R.
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1928	Reid, A.
1928	Sanderson, I.
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1929	Booker, C. G.
1929	Bullen, W. A.
1929	Callum, E. N.
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1929	Connolly, P. P. D.
1929	Cowan, J. A.
1929	Crawford, J.
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1929	Dogra, J. R.
1929	Drury, G. D.

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1929	McGregor, J. A.
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1929	Miller, A. A.
1929	Pearse, J. T. F.
1929	Ramdeholl, C.
1929	Robinson, Elizabeth J.
1929	Robinson, P. B.
1929	Rosenbloom, A.
1929	Row, C. K.
1929	Sewal, R. N.
1929	Shafi, A.
1929	Singh, H.
1929	Talwrn-Jones, G. A.
1929	Turner, H. N.
1929	Verghese, T.
1929	Wilson, S. P.
1930	Bagchi, K. W.
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1930	Caplan, J.
1930	Cathcart, J. A.
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1930	Chowdhary, D. S.
1930	Davey, T. H.
1930	Deacon, Ariel R. S.
1930	Dobbin, J. H.
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1930	Grant, S. C.
1930	Green, R.
1930	Gulatee, M. L.
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1930	Mohile, G. B.
1930	Mulligan, H. W.
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1930	O'Driscoll, F.
1930	Poh, C. J.
1930	Singh, M.
1930	Sumitra, L.
1930	Sung, J. K.

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1930	Verghese, G.
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1931	Behari, R.
1931	Bhambari, K. L.
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1931	Cutting, P. E. J.
1931	Fitzgerald, J. A. K.
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1931	Henry, A. F. X.
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1931	Tripp, Dorothy M. H.
1931	Ullah, S.
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1932	Adams, H. E. M.
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1932	Zau, F. D.
1933	Amirtha Nayagam, G. P. A.
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1934	Greson, L. P.
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IN MEMORIAM

The Editors regret to announce the death of Sir FREDERICK CHARLES BOWRING, J.P., D.L., which occurred on March 24th, 1936.

Sir Frederick was elected in 1924 to the General Committee of the Liverpool School of Tropical Medicine as a representative of the Shipowners' Association. On July 28th, 1926, he accepted the office of Chairman of the School, which position he held until April, 1934, when ill health compelled him to resign. He continued, however, to serve on the General Committee, of which he remained a member until the time of his death.

ANOPHELES FUNESTUS GILES AND *ANOPHELES LEESONI* EVANS IN HUMAN HABITATIONS AND OUTDOOR HAUNTS

BY

BOTHA DE MEILLON, D.Sc., F.R.E.S.

(*South African Institute for Medical Research, Johannesburg*)

(*Received for publication* 15 *November*, 1935)

Following the discovery of a method of differentiating between the females of *A. funestus* and *A. leesoni* by means of the examination of the eggs of the gravid female (De Meillon, 1934), an attempt was made to determine the part, if any, played by *leesoni* in the epidemiology of malaria. The method also provided us with a means of identifying with certainty such females as were caught out of doors.

For this purpose adults were captured in native huts and at the same time in outdoor haunts such as crevices, under banks, etc. Only such adults as contained almost fully-developed eggs were dissected for evidences of malaria infection, and their identity was established by the examination of the eggs. This work was done at Tzaneen, Northern Transvaal, during the months of October, 1933, to February, 1934. Males were identified by means of the palps.

A. funestus Giles from Human Habitations

Total identified		Glandular infections			Stomach infections		
♀♀	♂♂	No. dissected	No. positive	Percentage of infection	No. dissected	No. positive	Percentage of infection
281	65	281	35	12	275	9	3

A. funestus Giles from Outdoor Haunts

Total identified		Glandular infections			Stomach infections		
♀♀	♂♂	No. dissected	No. positive	Percentage of infection	No. dissected	No. positive	Percentage of infection
21	20	21	0	0	12	0	0

A. leesoni Evans from Human Habitations

Total identified		Glandular infections		Stomach infections	
♀♀	♂♂	No. dissected	No. positive	No. dissected	No. positive
1	0	1	0	1	0

A. leesoni Evans from Outdoor Haunts

Total identified		Glandular infections		Stomach infections	
♀♀	♂♂	No. dissected	No. positive	No. dissected	No. positive
15	34	15	0	10	0

The numbers from outdoor haunts are small, owing to the difficulty of finding adult anophelines in such places. Daytime hiding-places are innumerable, very often inaccessible, and scattered over a very large tract of country near the breeding-places. The figures relating to hut catches are, however, significant, since they show that in four months' collecting a single *leesoni* was captured. This substantiates our former finding regarding the absence of males from dwellings (De Meillon, 1933).

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HYPERKERATOSES AND DEPIGMENTATIONS IN BEJEL

BY

ELLIS H. HUDSON, M.D.

(*American Mission, Deir-ez-Zor, Syria*)

(*Received for publication 9 December*, 1935)

Bejel is the Arab name for the non-venereal and usually childhood-acquired syphilis of the semi-nomad bedouin villagers of the Middle Euphrates (Hudson, 1932, 1933, 1936*a*). Consideration of the results of clinical and serological studies of bejel in both 'sick' and 'well' components of the bedouin community has led us to the conclusion that over 90 per cent. of the adult population have—or have had—bejel (Hudson, 1936*b*). Though some bedouin individuals have had short courses of treatment, the disease in the community as a whole remains virtually untreated.

The purpose of this paper is to present cases and photographs illustrating two types of lesion met with in bejel. The principle interest of these syphilitic lesions lies in the fact that conditions which are apparently identical have been regarded in some quarters as manifestations of yaws.

HYPERKERATOSIS

Following the law that syphilitic lesions appear at the points of greatest trauma, there is a huge overgrowth of the epithelium of the plantar surfaces in the bedouin who is compelled to walk barefoot winter and summer. Though many of the towns-people have also had bejel, this hyperkeratosis of the feet is not found among them, because they always wear foot-covering.

The condition is manifested in two forms: either as foci of cornification at the points on the soles of the feet that bear the weight of the body, or as a diffuse thickening of the whole skin, which tends to form fissures in cold, wet weather, and to present a rough moth-eaten surface in dry weather.

Case 1 (Pl. I, fig. 1). Hussein, a bedouin villager, 45 years old, who had had bejel in childhood and whose Kahn was positive, complained of sore points on the soles of the feet which had been present for 12 years, and which made walking very painful. He had extreme thickening of the plantar skin, with fissuring along the margins of both heels, and very tender round points of cornification on each foot, located, as shown in the photograph, at the three weight-bearing points. The condition forced the patient to assume a limping sidewise gait.

Case 2 (Pl. I, fig. 2). Sabahi, a bedouin villager aged 35, who had had bejel at the age of 20 and whose Kahn was positive, had skin and bone lesions of syphilis. The fissuring along the margin of the heel, as shown in the photograph, was not one of his complaints.

Case 3 (Pl. I, fig. 3). Helal, a bedouin villager aged 40, who denied knowledge of bejel but whose Kahn was positive, complained of pain in the nose and throat, of undoubtedly syphilitic origin. He had syphilitic papules upon the skin and thickened and fissured soles of the feet. The photograph shows the points—indicated by the arrows—at which the patient has sewed up the fissures with bits of string.

Case 4 (Pl. I, fig. 4). Hassan, a bedouin villager aged 45, who had had bejel in childhood and whose Kahn was positive, complained chiefly of pain in the throat, which he had had for three months. He had had similar trouble in youth, and had then been cured by

mercury inhalations. He presented a large cicatrix involving the pharynx, soft palate and both tonsils, and he had a fresh ulcer in the left pharynx. The condition of the soles of his feet is shown in the photograph.

CASE 5 (Pl. II, fig. 1). Mash-hur, a bedouin villager aged 65, who had had bejel in childhood and whose Kahn was negative, came with a complaint not related to syphilis, but presented the extreme hyperkeratosis shown in the photograph.

CASE 6 (Pl. II, fig. 2). Aysha, a little bedouin girl aged 6, came first to the clinic in 1932, at the age of 4, with fresh bejel lesions of the mouth and with extreme hoarseness. In the following months she received bismuth injections at irregular intervals, and the lesions of the mucous membranes disappeared. About two years after the first bejel infection she returned to the clinic, complaining of the lesions shown in the photograph, a fissuring dermatitis of both plantar surfaces. She had similar lesions on the hands and arms.

Comment. The foci of cornification shown in Pl. I, fig. 1, and the symptoms presented by the patient, strongly resemble the lesions described as ' crab-yaws.' The lesions shown in Pl. I, figs. 2–4, and Pl. II, fig. 1, resemble closely the plantar hyperkeratosis described in yaws. We have no numerical statistics for these hyperkeratoses because they are so common in the barefooted bedouin as to be considered practically universal. The rainfall in the region of Deir-ez-Zor is low, averaging about 5 inches per annum, and the cold season is usually mild and short, comprising about three months. The thermometer may fall below freezing point at night, but snow falls rarely. It is in the cold, wet season that these lesions become in some cases sufficiently troublesome to lead the subject to seek treatment. If the cold season were more severe or the rainfall greater, this lesion of late bejel would undoubtedly be the source of more complaint on the part of the bedouin subject.

Hermans (1928), in his monograph on framboesia tropica, refers to the ' typical plantar and palmar keratosis ' in yaws. Fitzgerald and Dey (1931), describing the manifestations of chronic yaws, say of hyperkeratosis : ' Its essential feature is an overgrowth of the horny layer which sooner or later cracks in lines corresponding to the natural folds of the skin, exfoliation occurs, and the breaking down of tissue beneath the thickened epidermis gives rise to ulcers of considerable depth. . . . In performing a rapid survey of the population in an infected area . . . it is often useful to observe the skin around the back of the heel. This is seen to be unduly thickened and shows numbers of fine cracks extending slightly upwards around the sides. This is of great assistance in the diagnosis of latent cases.' Hyperkeratosis, they say, ' subsides every year during the dry season.'

Harris (1934), in India, names as one of the few peculiar and outstanding manifestations invariably presented by the patients (with yaws) ' very painful cracks in the soles of the feet and palms of the hands.' Lambert (1929), writing of yaws in the Southern Pacific, refers to a late manifestation of yaws called kakaca in Fiji, which ' consists in the formation of horny callosities on that part of the plantar surface which bears the weight of the body. . . . These callosities crack, producing deep sulci. Later, in a person who has much barefoot walking to do, the surface becomes worn having a punched-out or moth-eaten look.

In the wet weather the feet become painful and throb after a long walk ; or possibly when the sulci become infected. This condition when painful gives the native a characteristic gait as if walking on hard peas.

' More unusually one sees a dry scaly appearance of palmar surface of the hands, which may crack slightly and peel.'

Lacapère (1923), in Morocco, describes hyperkeratosing plantar and palmar lesions in cases of ' Arab syphilis,' and illustrates them in his Plate X. Hewer (1934), in the Southern Sudan, noted similar lesions of the feet and hands in a group of cases which he described as being either yaws or syphilis. He illustrates a ringworm form of palmar dyskeratosis similar to the plantar lesion which we show in Pl. II, fig. 2.

It has been said that such lesions are diagnostic of yaws, and certainly it is admitted that they are found most commonly in regions where yaws is endemic. Blacklock (1932), however, says that the differential point about these lesions of the feet is one which should be taken in conjunction with a consideration of the effects of pressure and injury, in causing localization of a syphilitic eruption. ' If the syphilitic,' he says, ' were compelled constantly to walk barefoot on rough ground, e.g., in the case of syphilitic native children, it appears highly probable that the lesions of the feet in such a syphilitic would present much the same characteristic appearance as that attributed to the feet lesions of yaws.'

This hypothesis of Blacklock is supported by our observations as shown in the accompanying photographs. Given the same conditions, viz., childhood acquisition, lack of treatment, and absence of foot-covering, syphilis and yaws produce similar hyperkeratotic plantar lesions. If the lesions of syphilis and yaws differ, it is a difference only in the degree of the severity of the lesions, due to the greater heat and more humid climate of the regions where yaws is endemic.

In bejel, as reported in yaws, these dyskeratoses are not quick to respond favourably to specific treatment.

DEPIGMENTATION

Another manifestation of bejel which appears not rarely among the bedouin villagers is a patchy depigmentation of the skin. This occurs sometimes in the extensive scars of the skin lesions in bejel, or about the hands and feet in areas unrelated to the syphilitic lesions. Case 7 (Pl. II, fig. 3), for example, shows the hands of one of these syphilitic bedouins. He stated that the loss of pigment came on gradually and had slowly extended to the condition shown in the photograph. The darker skin is the normal colour, and the depigmented areas are pink. The skin in the depigmented areas seems of normal texture, and if ever a bedouin complains of this condition it is solely on cosmetic grounds.

Occasionally, as in Case 8, this patchy depigmentation involves the whole body, producing a piebald condition. The circumstances of this case were as follows :

Case 8 (Pl. II, fig. 4). Khazna, a bedouin woman villager, about 50 years old, who had had bejel in childhood and whose Kahn was positive, complained of pains in various

joints and had limitation of motion in the elbows and knees. Her left knee was swollen, with some fluid in the joint. She had a syphilitic cicatrix in her pharynx, with destruction of the uvula. The white patches over her body first began to appear three years before, and had progressed to the condition shown in the photograph.

Occasionally the depigmented areas are definitely associated with syphilitic skin lesions, as illustrated by the following :

CASE 9 (Pl. II, fig. 5). Mudhi, a bedouin woman villager, about 35 years old, who had had bejel in childhood and whose Kahn was positive, complained of spots which had appeared at the sites of former ulcers. There were six or eight spots of irregular outline on the right arm, on the backs of both hands, on the lips and on the left foot. She also had the scar of a periostitis of the third and fourth metacarpals of the right hand.

In our experience, the depigmentation once established is permanent and is not affected by specific treatment.

Comment. Lacapère (1923) has described such depigmentations as these occurring among cases of ' Arab syphilis ' in Morocco. He illustrates (in his Plate XXI) a woman with a generalized depigmentation very similar to our Case 8.

Such lesions have been described as characteristic of yaws. For example, Stannus (1928) said that ' It was well known that some extensive ulcerative lesions of yaws when healed left unpigmented scars. Another lesion associated with loss of pigment, one first described by Ziemann, consisted of a patchy depigmentation about hands, wrists and feet and ankles. Its etiology was then unknown and he called it " Melung "; it was now considered to be due to yaws.'

It is obvious that these cases which we herewith report in bejel patients are of the same nature as those to which Stannus refers as manifestations of yaws. The conclusion that we draw is that syphilis when propagated under the same conditions as yaws, viz., childhood acquisition and lack of treatment, will produce in some instances depigmentations comparable to those found in regions of endemic yaws.

CONCLUSION

Hyperkeratoses and depigmentations are linked with epidemiological factors, and occur in syphilis when its epidemiology is similar to that of yaws. They do not constitute valid differential points as between syphilis and yaws.

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PLATE I

EXPLANATION OF PLATE I

Fig. 1. Showing the condition of the feet of Case 1.

Fig. 2. Showing the fissuring along the margin of the heel of Case 2.

Fig. 3. Showing the fissured feet of Case 3. The arrows indicate the points at which the patient had sewed up the fissures with string.

Fig. 4. Showing the condition of the soles of the feet of Case 4.

PLATE I

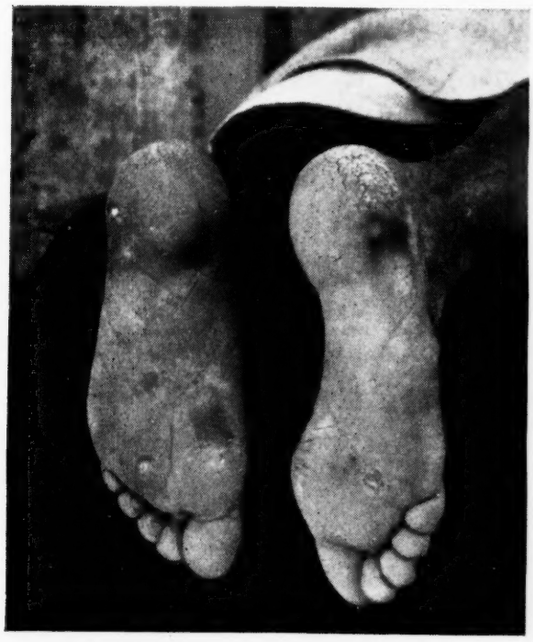


FIG. 1.

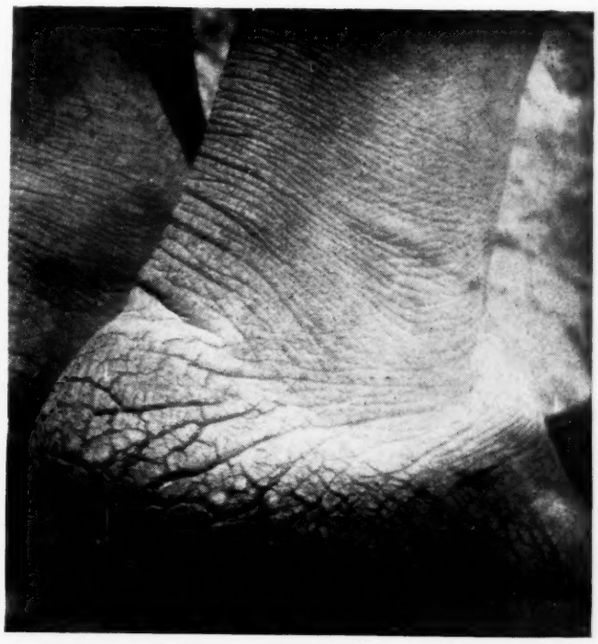


FIG. 2.

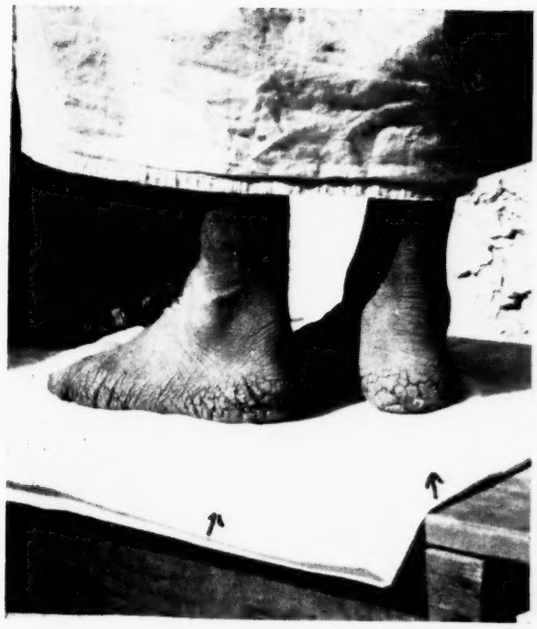


FIG. 3.



FIG. 4.

H. R. Grubb, Ltd., Poplar Walk, Croydon.

EXPLANATION OF PLATE II

Fig. 1. Showing the extreme hyperkeratosis of Case 5.

Fig. 2. Showing the fissuring dermatitis of both plantar surfaces of Case 6.

Fig. 3. Showing the patchy depigmentation of the skin of the hands of Case 7.

Fig. 4. Showing the patchy depigmentation of the skin of the body of Case 8.

Fig. 5. Showing spots which had occurred on the sites of former ulcers of Case 9.



FIG. 1.



FIG. 2.



FIG. 3.



FIG. 4.



FIG. 5.

H. R. Grubb, Ltd., Poplar Walk, Croydon.

A STUDY OF LABORATORY-BRED *ANOPHELES MACULIPENNIS* var. *ATROPARVUS*, WITH SPECIAL REFERENCE TO EGG-LAYING

BY

P. G. SHUTE

(*From the Ministry of Health's Malaria Laboratory, Epsom*)

(*Received for publication* 14 *December*, 1935)

It is well known that among the six recorded varieties of *Anopheles maculipennis* only one, *atroparvus*, breeds readily in captivity.

During the past two years I have maintained a genetically pure strain of this variety in the laboratory, and have found that, providing the temperature and humidity are of a summer character, breeding continues uninterruptedly throughout the winter months.

Having developed a technique whereby a large stock of newly bred insects is always available, I have carried out experiments to obtain information on the length of life of the adult insects and their powers of egg-laying.

It was realized that some variation may occur at different seasons of the year, despite the fact that a constant temperature of 75° F. was maintained in the laboratory. For this reason it was decided to divide the experiment into three parts, one in the spring, one in the summer and one in the winter.

The female adults were bred out into a large cage and were left undisturbed with an equal number of newly bred males for 24 hours. The following day all the males were removed, and the females were fed on animal blood. On no occasion did any of the females used in the experiments have access to males after a first batch of eggs was laid.

As each adult, after having a blood meal, showed developing ovaries, each was removed to a small glass cylinder with a small petri-dish of water, where it remained until it had laid its eggs. Having laid a batch of eggs, it was removed, given a meal of blood, and then, usually 24 hours later, when developing eggs could be seen, it was again put back into the glass cylinder, where it remained until a further batch of eggs was laid.

FIRST EXPERIMENT

Started 11.5.34 ; ended 9.7.34

58	(100	per cent.)	laid	1	batch	of	eggs
40	(69	„)	survived and laid	2	batches	of	eggs
34	(59	„)	„ „	3	„	„	
30	(52	„)	„ „	4	„	„	
25	(43	„)	„ „	5	„	„	
20	(34	„)	„ „	6	„	„	
18	(31	„)	„ „	7	„	„	
14	(24	„)	„ „	8	„	„	
10	(17	„)	„ „	9	„	„	
10	(17	„)	„ „	10	„	„	
5	(9	„)	„ „	11	„	„	
4	(7	„)	„ „	12	„	„	
3	(5	„)	„ „	13	„	„	
2	(3	„)	„ „	14	„	„	
2	(3	„)	„ „	15	„	„	

The above 58 insects laid 275 batches of eggs over a period of 59 days.

Twenty-seven (10 per cent.) of the batches of eggs failed to hatch. The two insects which survived long enough to lay 15 batches of eggs still showed numerous spermatozoa in the spermathecae.

SECOND EXPERIMENT

Started 5.9.34 ; ended 21.11.34

58	(100	per cent.)	laid	one	batch	of	eggs
48	(83	„)	survived and laid	2	batches	of	eggs
35	(60	„)	„ „	3	„	„	
26	(45	„)	„ „	4	„	„	
19	(33	„)	„ „	5	„	„	
14	(24	„)	„ „	6	„	„	
12	(21	„)	„ „	7	„	„	
10	(17	„)	„ „	8	„	„	
6	(10	„)	„ „	9	„	„	
6	(10	„)	„ „	10	„	„	
5	(9	„)	„ „	11	„	„	
4	(7	„)	„ „	12	„	„	
3	(5	„)	„ „	13	„	„	
2	(3	„)	„ „	14	„	„	
2	(3	„)	„ „	15	„	„	
1	(2	„)	„ „	16	„	„	
1	(2	„)	„ „	17	„	„	

The above 58 insects laid 252 batches of eggs over a period of 78 days. Thirty-three (13 per cent.) of the batches of eggs failed to hatch.

THIRD EXPERIMENT

Started 22.1.35 ; ended 14.4.35

48 (100 per cent.) laid one batch of eggs
40 (83 „) survived and laid 2 batches of eggs
32 (68 „) „ „ 3 „ „
24 (50 „) „ „ 4 „ „
18 (38 „) „ „ 5 „ „
16 (33 „) „ „ 6 „ „
12 (25 „) „ „ 7 „ „
8 (17 „) „ „ 8 „ „
4 (8 „) „ „ 9 „ „
2 (4 „) „ „ 10 „ „
1 (2 „) „ „ 11 „ „
1 (2 „) „ „ 12 „ „

The above 48 insects laid 206 batches of eggs over a period of 82 days. Seven (3 per cent.) of the batches of eggs failed to hatch.

Percentage of survival in the three experiments

Series	1st experiment May-July Per cent.	2nd experiment Sept.-Nov. Per cent.	3rd experiment Jan.-April Per cent.
1	100	100	100
2	69	83	83
3	59	60	68
4	52	45	50
5	43	33	38
6	34	24	33
7	31	21	25
8	24	17	17
9	17	10	8
10	17	10	4
11	9	9	2
12	7	7	2
13	5	5	—
14	3	3	—
15	3	3	—
16	—	2	—
17	—	2	—

Percentage of the batches of eggs which hatched in the three experiments

Series	1st experiment May-July		2nd experiment Sept.-Nov.		3rd experiment Jan.-April	
	Batches of eggs	No. of batches hatched	Batches of eggs	No. of batches hatched	Batches of eggs	No. of batches hatched
		Per cent.		Per cent.		Per cent.
1	58	55 (95)	58	53 (91)	48	48 (100)
2	40	39 (97)	48	44 (92)	40	38 (95)
3	34	32 (94)	35	31 (89)	32	31 (97)
4	30	27 (90)	26	23 (89)	24	24 (100)
5	25	22 (88)	19	16 (84)	18	18 (100)
6	20	18 (90)	14	11 (78)	16	16 (100)
7	18	14 (78)	13	10 (77)	12	12 (100)
8	14	11 (79)	10	7 (70)	8	7 (88)
9	10	9 (90)	6	6 (100)	4	4 (100)
10	10	7 (70)	6	5 (99)	2	1 (50)
11	5	4 (80)	5	4 (80)	1	1 (100)
12	4	3 (75)	4	3 (75)	1	1 (100)
13	3	3 (100)	3	2 (66)	—	—
14	2	2 (100)	2	2 (100)	—	—
15	2	2 (100)	2	1 (50)	—	—
16	—	—	1	1 (100)	—	—
17	—	—	1	1 (100)	—	—

GENERAL INFERENCE

During the first three egg-layings the death-rate was considerably higher in the May-June series, particularly so after the first egg-laying.

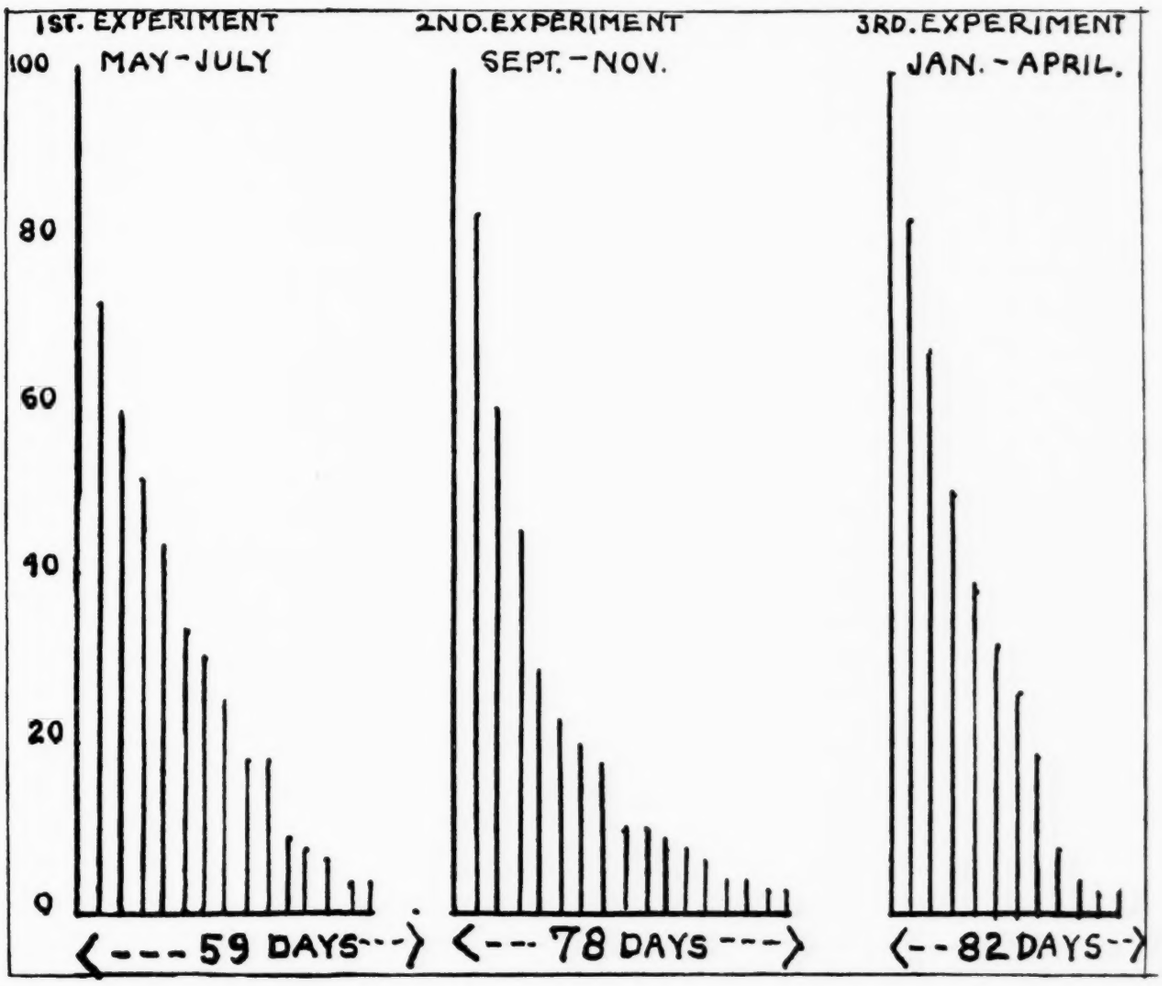
Rather curiously, the rate of survival is much greater in this series following the 3rd egg-laying and continuing until the 10th than is the case in the other two series, particularly so in the September-November series. Yet, following the 10th egg-laying and continuing until the 15th, the rate of survival in the September-November series again equals the May-June series.

Every opportunity was taken to dissect those insects which died during the experiment. As far as could be ascertained every insect was fertilized ; in no case was a negative spermatheca found. It may therefore be interesting to note that the highest percentage of eggs which hatched belong to the January-April series, the next highest in the May-July series, and the lowest in the September-November series.

It would therefore appear to suggest that, despite the fact that an even temperature of 75° F. was maintained throughout the experiments, with an

TABLE

Showing length of life and number of batches of eggs laid



accompanying relatively high degree of humidity, nature seems to have asserted itself in some way as regards the fecundity of the eggs at the various seasons of the year, being highest in the spring and lowest in the late autumn.

OBSERVATIONS AND CONCLUSIONS

Time of egg-laying

In every case egg-laying took place at night. Usually it takes about three hours for the newly laid cream-coloured eggs to change to the dark-grey colour. On arrival at the laboratory about 8.30 a.m., eggs which had been laid the previous night had already changed colour ; hence it would appear that egg-laying takes place either very late at night or very early in the morning.

Fertilization

Fertilization takes place at all hours of the day in the laboratory. The females are fertilized while resting on the roof or sides of the cage. A male and female of var. *atroparvus* confined in a very small area readily copulate, even in a 5 in. $\times \frac{1}{2}$ in. test-tube.

Feeding habits

An outstanding feature of the experiment was that immediately after oviposition the insects would feed readily, often biting a rabbit within two or three minutes of being put into the cage.

In some cases adults with ripe ovaries refrained from egg-laying for four or five days, but for the most part refused a meal of blood during this interval.

Ovary development

Usually one blood meal was sufficient to bring about complete development and actual egg-laying. Rarely it happened that two or more blood meals were required.

Some examples may be quoted :

A. Laid eggs night of 28.5.34. Fed on blood morning of 29.5.34. Laid eggs night of 31.5.34.

B. Laid eggs night of 2.6.34. Fed on blood morning of 3.6.34. Laid eggs night of 6.6.34.

C. Laid eggs night of 2.6.34. Fed on blood morning of 3.6.34. Laid eggs night of 7.6.34.

Those insects which survived long enough to lay more than ten batches of eggs laid approximately 2,500 eggs. As has already been noted, the spermathecae of these long-lived insects still contained spermatozoa, which proves that once a female is fertilized the spermatheca never becomes negative.

As showing the importance of killing adult *maculipennis* in houses, previous observations to the effect that a single heavily infected insect may continue to harbour sporozoites after having fed 30 times (Christophers and Missiroli, 1933) can now be supplemented by the knowledge that the insect can live at a temperature of 75° F. for $2\frac{1}{2}$ months, during which time it may lay more than 2,500 fertile eggs.

REFERENCE

CHRISTOPHERS, S. R., and MISSIROLI, A. (1933). Report on housing and malaria. *Quart. Bull. Hlth. Org. L. o. N.*, **2**, 377.

A SURVEY OF HEALTH WORK IN TESO, UGANDA

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INTRODUCTION

A population of 300,000 natives and a European medical and sanitary staff of three present a difficulty not met with in civilized countries. It is obvious that curative medicine alone can have no sociological effect on the district, and the development of health work must offer the best hope of bringing the greatest good to the greatest number. Teso is only one district of Uganda, in all parts of which the same problem has to be faced. It was for this reason that an enunciation of departmental policy (Annual Report of the Medical Department) was made in 1934, and it is the object of this paper to illustrate the application of this policy to a particular area.

PART I

DATA OF MEDICAL AND SANITARY IMPORTANCE

A. *Topography*

The district of Teso comprises some 4,052 square miles, and occupies the northern portion of the Eastern Province of Uganda. It lies roughly in longitude 34·0° E. and latitude 2·0° N., and is about 3,500 feet above sea level. The population numbers 295,000, the vast majority being natives of the local tribe, Iteso ; in addition, there are a few dozen Europeans and several hundred Asiatics.

Although Teso is, on the whole, hotter than most other districts in the Protectorate, its climate shows distinct variations, tending to be dry and torrid in the north-east and more equable in the south-west. Reference to the map explains this difference, which is due partly to the lakes in the west and partly to the arid condition of the Karamoja district, adjacent on the north-east.



MAP OF TESO.

Normally the long rains start towards the end of March and cease in June ; lighter rains fall from August until November. The beginning of the year is always hot, dry and windy, and these features are accentuated as one approaches the Ussuk and Amuria Sazas (counties). This climate is favourable for the cultivation of millet, supplemented by smaller crops of sweet potato, manioc and ground-nuts. Of recent years cotton-growing has increased enormously, and this crop is of great local economic importance.

Dual administration is in force, the object being to foster native self-government without imposing sweeping changes that might arouse suspicion and resentment in the uneducated African. It will thus be appreciated that health work, outside of townships, must be by persuasion rather than legislation, and that due regard must be paid to tribal beliefs and customs.

Native administration is by chiefs, elected and acting with Government approval. The district is divided into eight ' Sazas,' or counties ; each of these consists of a number of ' Gombololas,' and further sub-division into ' Mulukas ' and ' Mutongoles ' is made, each with their appropriate minor chiefs. The term ' village ' is a loose one and may comprise dwellings scattered over an area of five miles square, as one compound is rarely built within 200 yards of another. This point is of importance, as adequate inspection of a single ' village ' may mean three or four days' walking.

Communications are good ; most native dwellings lie within seven miles of a road or track passable to motor-cars. Sanitary control of native movements, however, is almost impossible, as the African travels from one place to another by bush paths and, in addition, the lakes offer an easy means of access by canoe.

B. *Townships*

Apart from Soroti, which is the administrative headquarters, there are eleven townships in Teso ; most of these consist of a single row of trading premises with residences, on each side of a main road, and vary in size according to the amount of native trade. The occupant is usually an Indian, though a few Goans and Arabs compete ; generally, the occupants' standard of living is low, and the condition of these townships approximates to the European idea of slum property.

Soroti itself is wealthier and is rapidly changing from a row of heterogeneous buildings into a planned and orderly township. No buildings of corrugated iron remain here, but other structural faults are disappearing less rapidly ; overbuilding, sub-division of small plots, insufficient drainage of compounds and badly constructed latrines are faults which it is difficult to eradicate in existing buildings, but which are obviated in those recently erected.

Refuse is collected and burnt in all townships, and excreta are disposed of by trenching in the larger, and by pit latrines in the smaller, centres; these services are rendered by Government-paid township labour.

A township comprises an area varying from a half to one and a half miles in radius, usually measured from the market, and invariably contains a number of native huts for which no sanitary arrangements are, as yet, made. These huts are not allowed on the surveyed plots of the townships, i.e., in proximity to the permanent buildings. There is a central township authority for the whole district, consisting of the District Commissioner, the District Medical Officer, an engineer from the Public Works Department, and one private (Indian) member for Soroti only. Sanitary measures in the townships, therefore, are performed on behalf of the township authority and not, as is the case in rural areas, with the co-operation of the chiefs.

C. *Factories and Labour Camps*

In Teso district cotton-ginning is the only mechanical industry, and the ginneries themselves are under supervision from a central Factories Board. The housing of labour, however, is one of the sanitary problems which concern the Medical Department, and conditions obtaining in most camps leave much to be desired at present. Unskilled labour is cheap and plentiful, and a few sick workmen can always be replaced by imported or locally recruited individuals ; is it any wonder, then, that employers attach less importance than they ought to the living conditions of their labourers ?

D. *Native Dwellings*

The original Teso hut, now but rarely seen, was a high ' sugar-loaf ' erection of flounced thatch, descending from a central pole to the ground ; there was no separate wall, and the doorway was so small as to necessitate crawling in and out on hands and knees. The size of this type of hut was usually greater than those at present in use, and it accommodated, not only the owner and his families, but his animals, food-stores and kitchen.

Nowadays, a wattle and daub hut is universal, grass-roofed and round or square, according to the owner's taste ; the size varies greatly, averaging a diameter of 20 feet among the poorer peasants. The old, flounced type of thatching has died out and smooth thatching with broad-bladed grass is the rule ; this is regrettable, as smooth thatching usually lasts less than a year and looks dirty and dilapidated in a very short time. Extended eaves, which protect the mud walls against weathering, are popular but seen only in those parts where straight poles are easily come by, i.e., in the south and west. Huts are built without windows or ventilation of any kind, and, though cool, are invariably dark, even on the brightest day. Sheep, goats and fowls are housed with the family ; cattle are placed in open enclosures walled with growing cactus or heaped-up thorn bushes. Calves are often seen tied to a verandah-pole by day and taken into the hut at night.

Never in a single instance have the authors seen kitchen and latrine

accommodation among the Iteso before the advent of alien influence. Such accessories were, in fact, unknown and undesired ; cooking is always done in the living huts, and excreta deposited at random in the bush.

It is now becoming the custom for a mud house to increase in size, *pari passu*, with the owner's estimate of his own importance ; unfortunately, however, the most elementary rules of hygiene are as conspicuously absent in the larger as in the smaller dwellings.

E. *Tribal Customs and Beliefs of Sanitary Importance*

1. *The Iteso* belong to the Eastern half-Hamites and are closely related by language and custom with the Masai and similar tribes. Like these, they were originally cattle-owning nomads, but the Iteso have now settled down and, while cattle are still the standard of wealth and social position, cultivation is now an important part of tribal life.

2. *Dwellings.* Polygamy is an accepted feature in tribal life, and each wife occupies a separate hut in the compound of the master ; often the latter has no hut of his own, but moves from one wife to another in turn. As the children reach puberty (but sometimes earlier) those from each wife build their own hut in the compound with the assistance of their father ; the sexes are often separated. With increase in the family fortunes and numbers, the compound tends both to increase in size and to become crowded with huts, all of an insanitary type. Doorways are low and windows or open ventilation are still ' tabu ' with the primitive Etesot, who regard such innovations as possible means of access for ' the evil eye,' mosquitoes and other unwelcome visitors.

A most important custom is the almost universal one of vacating the hut in which a person has died ; to persuade the native to build an elaborate and relatively costly dwelling, which may have to be vacated very shortly owing to a death, is not a practicable measure in Teso. It would be difficult to over-estimate the importance of this observation.

3. *Disposal of Excreta.* Local custom is strongly against the habitual use of any definite place for this purpose, and defaecation is performed in a different patch of bush from day to day. This prejudice is probably an imported one, though it may be connected with the Iteso's original nomadic habit. Whatever its origin, however, it now forms an obstacle to the introduction of faecal disposal methods. The reason given by the Iteso for their dislike of habitually using one spot for defaecation (and hence of latrines) is the following : ' An enemy would be able to come during the night and put a poisonous powder in that place ; this powder has the property of poisoning a man through his anus if he revisits the spot.' This explanation, it is almost needless to add, has no scientific foundation.

It is to be noted that the Etesot does not defaecate near a water-supply nor over food crops; hence a valuable measure in local sanitation is the planting of root-crops in areas threatened by faecal pollution.

4. *Food and Water.* It must briefly be mentioned that the principal foodstuff is millet, from which grain beer is also manufactured in every household. This beer is the sole breakfast diet in many parts, and is generally partaken of freely by all, from the infant upwards. In the author's opinion it is a valuable preventive of scurvy and other deficiency diseases, which tend to appear at the end of the dry season, when beer is scarce (Loewenthal, 1935). As is natural in a country subject to drought, there are ' tabus ' connected with water, and light-hearted interference with supplies is resented.

5. *Birth Customs.* Women are accustomed to work in the fields until labour begins ; afterwards, a fortnight's rest is the rule. Contrary to the belief obtaining in civilized countries, the process of childbirth is not generally any easier than that seen in the white races, and (although this would be difficult to prove) maternal mortality and morbidity rates are probably higher than in Europe. The parturient mother is attended by the wise woman of the village, and numerous interesting customs are observed ; labour usually takes place in a hut with the door closed, a smoky fire providing fitful illumination. In one district, however, it is insisted that the door remain open—probably a symbolic transference from the desired easy delivery. The infant is, in most cases, kept at the breast until it is three or four years old, though after the first year supplementary diet is given.

6. *Burial.* On the death of an important person the corpse is kept in the hut for two or three days, during which time relatives at a distance take the opportunity of arriving, and, incidentally, the corpse that of putrefying. Clouds of flies surround it, laying their eggs or larvae, and if the grave should be a shallow one thousands of fresh disease-carriers may thus be produced. The grave is dug in either the hut or the compound, and varies in depth according to the nature of the soil—it is usually from two to six feet ; and the body is invariably buried in a cerement of new calico.

Cannibalism of a ' mystic ' type is not uncommon even to-day, and is often associated with strange rites. After burial of the remains, a fresh supply of beer is brewed, and, on its fermentation three weeks later, a beer party is summoned.

7. *Miscellaneous.* Tribal customs are changing or disappearing rapidly as the result of Christianity, trade and increasing communications. The advent of ordered legislation, the plough, the bicycle, currency and the motor-'bus is already producing its effects, and rural sanitation must adjust itself to the varying proportions of new and old customs that obtain in different parts of Teso.

F. *Water-Supplies*

That part of the population which inhabits the fringes of the lakes and swamps naturally draws its water from these sources. Apart from the common danger of crocodiles, this method has other disadvantages : the smaller swamps are liable to dry up in the hot season, forcing the native to dig for water for human and animal needs ; the condition of such supplies is, of course, dangerous

to health ; and residence nearby is invariably associated with malarial hyper-infection.

Sub-soil springs, flowing on ironstone or laterite, have been exposed by natives in many parts of Teso and form the most valuable source of water. As a rule it is not customary to draw water as it issues from the ground ; a shallow pool is prepared some yards below, and water is got by scooping with a calabash, the supernatant scum having first been skilfully brushed aside. In the more copious supplies certain places down-stream are set aside for bathing and the washing of clothes (if any), and others for cattle. In certain areas cattle and humans drink from the same pool, possibly because the annual diminution in the flow of the spring causes the disappearance of pools lower down. Many of these springs fail completely during the dry season, and it is then that recourse must be had to artificial water-holes or to a temporary migration to the lake shores and swamps.

Water-holes are especially frequent in the relatively dry Ussuk country ; they are dug during the rains and serve to collect surface water. The condition of their contents at the end of the dry season is appalling, and they form a means for the dissemination of guinea-worm, among other diseases.

There is thus no safe natural or native-made water-supply in Teso, and improvements in this direction obviously form an important part of the health programme.

G. *Diseases*

As will be obvious from reading the above account, dirt, overcrowding, poor housing and diet and impure water must be the cause of numerous endemic diseases. Plague, dysentery, smallpox and cerebro-spinal fever occur to a greater or lesser extent from time to time ; yaws, helminthiasis and deficiency diseases constantly affect a certain proportion of the population, and every adult native has developed a tolerance to the malaria parasite. Trachoma and the venereal diseases, too, are very prevalent, and tropical ulcer is one of the conditions most frequently requiring medical assistance.

The high incidence of the affections detailed above compared with that of the remaining conditions which a practitioner in this country has to treat, is a powerful argument in favour of preventive medicine.

PART II

ORGANIZATION OF THE HEALTH SERVICE

The European staff comprises a District Medical Officer, a nursing sister and a sanitary inspector. The first two must needs devote a large part of their time to curative medicine, but the last is in direct charge of a Health Office and a sanitary personnel. An Indian sub-assistant surgeon relieves the District

Medical Officer of a great amount of work in connection with Asiatic and native sick, and with store accountancy.

The African staff consists of a medical and a sanitary division. At the head of the former is a senior African medical assistant—a licensed medical practitioner—who, as well as acting as house surgeon to Soroti Native Hospital, pays regular visits to sub-dispensaries and is being trained in rural-sanitation work. It is probable that large-scale health campaigns (e.g., anti-yaws) will in future be conducted by him under advice from the District Medical Officer. The remainder of the medical division consists of trained male and female attendants (dressers), who are posted to Soroti Hospital and four sub-dispensaries ; they have been taught sick-nursing, and various individuals are able to give intravenous injections, dispense medicines, and diagnose and treat the commoner complaints (Webb, 1934). Two fully-trained African midwives help the nursing sister in the important Ante-Natal and Child Welfare Clinics, and one of these women, posted to Serere Health Centre, holds a clinic for women and children daily, as well as attending an average of ten confinements a month.

A trained laboratory assistant and two trained mosquito-searchers fulfil rather specialized functions : the former, as well as conducting routine pathological investigations for the Hospital, is available for the examination of slides from suspected outbreaks of plague and other infectious diseases ; the mosquito-searchers are occupied in checking up the results of anti-malarial measures in Soroti township, i.e., their labours are confined essentially to work on behalf of the non-African population.

Six sanitary orderlies are employed in the district, and of these five have been trained by one of the present authors (R.C.D.H.). Township work in Soroti does not call for any especial mention here, but four sanitary orderlies who are stationed in rural areas have very specialized functions which require a more detailed description. It is felt that the future of rural sanitation depends largely on these men and their successors, constituting as they do a link between the European staff and the individual peasant. According to European standards they may seem ignorant ; that they will eventually be supplanted by other Africans with a more generous education and special training is inevitable ; but with all their shortcomings they have proved reliable, enthusiastic and reasonably intelligent, and they represent the beginning of an organization which must one day play an important part in the health of many districts.

These four sanitary orderlies were locally trained, the language of instruction being Kiswahili (the *lingua franca* of East Africa). Each is posted to the headquarters of a Saza (or county) and lives in a model compound, the building of which he has himself supervised ; these model dwellings are intended to serve as an example to neighbours, and, in one instance, a sanitary orderly's compound is part of a health centre.

The routine work of a sanitary orderly is mainly the regular inspection of native ' villages,' outlying townships and native administration prisons.

In addition, he reports promptly cases of suspected infectious disease and accompanies these reports with relevant specimens. He supervises the building of model compounds to be occupied by African Government servants or by private individuals. His work is checked by frequent visits from the European staff and by the weekly reports which he sends in, the contents of which are dissected and entered in the appropriate records in the health office.

Urgent messages are sent to and from the outside sanitary orderlies by cycle 'runners' provided by the native administration; in addition, each sanitary orderly maintains a cycle which gives him access to every part of his Saza; the average monthly cycle-running of these men is about 800 miles. Supervision is effected by the District Medical Officer's car and a transport van in charge of the sanitary inspector, the latter car being useful for the conveyance of stores. Recently a motor ambulance has been provided by the native administration, and this vehicle, apart from special journeys for the transport of urgent cases, pays regular visits every week to the four sub-dispensaries.

Records of purely sanitary importance are compiled in the Health Office; about 50 files in all are kept and provide immediate information about the condition of all townships, markets, ginneries, schools and anti-malarial works, as well as those of villages and water-supplies on which work has been done. To have these records with their accompanying plans always accurate to date entails the expenditure of much time, but, until clerks adequately trained for this specialized work are available, it must devolve on the sanitary inspector.

PART III

PRESENT ACTIVITIES OF THE HEALTH SERVICE

A. *Townships*

Every township building in the district is inspected regularly by a European, special attention being paid to the lay-out, structural condition and state of cleanliness; sanitary orderlies also inspect once weekly such townships as lie within their area, and supervise the work of the paid labour in sweeping, grass-cutting, burning of refuse and disposal of night-soil. In several of the minor townships short grass has been planted where the bush has been cleared.

A complete survey of every permanent building in the district, with scale plans, is the aim; such work is well advanced in Soroti, where structural alterations to conform with legislation are being insisted on, and, throughout the district in general, the renewal of leases is subject to conforming with the recommendations of the Medical Department to the township authority. When such a survey has been completed discrepancies are always found, and it is sometimes laughable to compare the plans of the buildings as they stand with

those originally passed by the township authority. Proceedings for remedying both structural defects and general nuisances are much the same as obtain in England.

Native dwellings present a particular problem in Soroti, the leading township of the district, and, subject to two years' notice, will in future have to be confined to certain areas and conform with definite structural and sanitary requirements.

B. *Factories and Labour Camps*

During the ginning season, special inspections are undertaken, and at other times vacant camps are seen approximately once a month.

Before the season opens a complete inspection of ginnery sanitation and labour camps is made, and the granting of a license to gin is conditional on these being adequate.

C. *Schools*

Schools of various grades throughout the district are inspected regularly, and, when time permits, the pupils themselves are examined for gross signs of ill-health. This work is of great importance, and should be extended appreciably in the future. It is usually possible to have sanitary defects remedied following a consultation with the European managers of these schools.

D. *Water-Supplies*

The drilling section of the Public Works Department has recently installed several bore-holes (three with windmill pumps) in Soroti and the drier parts of the district, and the native administration proposes to supplement these supplies with shallow bore-holes fitted with hand pumps. The protection and amplification of supplies derived from sub-soil springs is one of the activities of the Medical Department ; funds are provided by the native administration, and the work has been organized in the following manner :—

A gang of four specially trained men is employed, under the direct supervision of the sanitary inspector. They make composite cement blocks at Soroti, and do the actual building work after the total number of blocks required has been transported to the selected site. To avoid excessive cost, blocks are made by pouring cement into specially constructed moulds which hold either two or four standard-size burnt bricks ; to use locally made bricks without such binding and facing would not be feasible, as they are reduced to pulp in a few days when in contact with water. These blocks are thus either 9 in. × 9 in. × 6 in. or 9 in. × 9 in. × 3 in. ; the former variety is used for the front facing of the impounded spring, and the latter, placed edge to edge, make a 3-in. wall at the sides and back.

Foundations for the walls of these impounded springs are necessarily built on the laterite or ironstone strata, as excavation of these might lead to cracking and hence to disappearance of the spring. Ferrocrete is used in making the

foundations and cementing the blocks together. The usual shape of the completed tank is an oblong, and delivery is effected through a 2-in. pipe cemented into one of the blocks in the front wall ; to reach this level the water has to rise 2 ft. or more, and on its way up filters through a layer of coarse gravel and sand. An overflow pipe is built into the top row of blocks in a side wall, and discharges into a sub-soil drain. Finally the tank is roofed with poles and earth, the sides are banked up, short grass is planted over the whole, and a hedge is put in to give protection against cattle. Large stones are placed under the outflow to avoid damage to the foundations, and the water is led off into a clean channel. Future improvements may take the form of a washing-place for clothes and a cattle trough in the bed of the channel.

Apart from the immediate gain in procuring a relatively pure water-supply, this work furthers the health programme in other ways ; gratitude, for instance, may make the African more tolerant of other health measures whose purpose is obscure to him, especially when such measures are introduced by the very officials who supervised the impounding of a spring. It is noted that the natives are now building new compounds near the improved supplies, and it is possible that in the future definite settlements will arise at such places, and the control of rural sanitation will thus make significant progress.

E. *Rural Sanitation*

The sanitary orderly spends at least four days a week inspecting native dwellings in definite parts of his area. He makes notes of the state of cleanliness of the compounds, height of doors, lighting and ventilation, and presence or absence of kitchens and latrines. He is accompanied by the Muluka chief, to whom he makes his recommendations. The minimum standard aimed at is as follows :—

Compound to be free of food crops and long grass for at least 20 yards outside any dwelling.

Living-huts to have a doorway at least 6 ft. in height, a 9-in. air-space between the 7-ft. mud wall and the roof, and four small windows, near the top of the wall, to admit daylight.

Floors to be either of rammed murram or of cow-dung freshly spread once weekly.

A kitchen to be provided for every family (this is the measure most difficult to introduce).

A pit latrine of 10 to 20 ft. in depth for every compound, and a small mud and thatch superstructure with adequate ventilation to be built over this.

Animals to be housed separately from humans.

Grain-stores to be as nearly rat-proof as possible.

Should a person wish to build a new hut, he is encouraged to consult the sanitary orderly, who will make the necessary measurements and supervise the building.

During his tours of rural inspection, the sanitary orderly notes the whereabouts of all water-supplies used by humans and cattle, emphasizes the necessity of keeping the surroundings clean, and reports those which he thinks are suitable for reconstruction.

As often as possible one of the European staff attends the fortnightly chiefs' councils and gives an address on points of hygiene of topical interest, stressing sanitation of dwellings and water-supplies, and ante-natal work; interest is usually gratifying, but addresses of this kind must be given frequently for results to ensue. The sanitary orderly, too, makes a point of attending the 'barazas' in his area, and thus keeps the sanitation question before the minds of the chiefs.

In addition to his routine work, the sanitary orderly visits all outbreaks of suspected infectious disease in his area, and sees the bodies of those who have died from an unknown cause; they can be relied on to report promptly cases of suspected plague, cerebro-spinal fever, smallpox and measles, and it is remarkable how rarely their diagnoses are mistaken.

Vaccinations are performed Saza by Saza, one such area taking approximately a year to complete; thus, when vaccination of the whole district has been done methodically, it will be time to start again in the Saza first worked over.

F. *Sub-Dispensaries and Health Centres*

Originally intended as a centre for the treatment of disease in outlying parts of the district, the sub-dispensary is now changing its character. The use of an ambulance centralizes the work, and elaborate wards and operating theatres will not, in future, be required; as regards the treatment of urgent cases, the sub-dispensaries are tending to become collecting stations, where a rough diagnosis can be made and first-aid treatment given while the ambulance is summoned. The treatment of out-patients is conducted by the medical attendants described in Part II, and an average of one hundred is seen daily at each of the four sub-dispensaries.

With the new policy, the sub-dispensary is gradually becoming a health centre: special attention is paid to ante-natal work and the care of children, and parents are being encouraged to bring their children for regular examination, even when they are apparently in good health. Serere is an example of such a centre: here a midwife is posted and holds a daily clinic for ante-natal and infant cases, and the District Medical Officer and nursing sister attend on a specified day each week for the same purpose. The following buildings constitute Serere Health Centre:—

Permanent: 1. One block containing a central consulting room and two wings; one of these consists of an examination room, douche and dressing-room and labour room, and is in charge of the midwife. The other wing is given over to work connected with out-patients, i.e., dressings, injections and dispensing.

2. A block of four side-by-side rooms accommodating the male staff of the sub-dispensary.

Temporary : 1. Sick lines of round huts, with kitchen and latrines, for serious cases awaiting transport to Soroti.

2. Kitchen, wash-place and latrines for the resident staff.

3. Model compounds for the midwife, female nurse and sanitary orderly.

4. A lying-in ward to accommodate six women and infants, with its own kitchen, wash-place and latrines.

It is proposed to extend this health centre gradually, adding from time to time such items as an incinerator, a clothes' disinfector and model grain-stores, all to be made of locally procurable materials. In this way the native will be taught how much can be done with the materials at his disposal. The centre is situated on the four corners of an important cross-roads and cannot fail to meet the eye of the passer-by ; improvements in clearing and planting of short grass and the laying-out of borders and flower-beds are measures which will add to the attractiveness of the centre and thus increase its usefulness.

Each sub-dispensary is the centre of four or five injection posts, which a dresser visits by cycle on a specified day each week, treating such conditions as yaws and syphilis. Injections are popular with the natives, and this spread of curative work is designed to win their confidence and thus to facilitate the introduction of health work into the remoter parts of the district.

G. *Anti-Malarial Work*

This work is at present confined to Soroti, and an anti-malarial survey with recommendations has been done at Serere Agricultural Experiment Station ; but the possibility of extending similar activities to other townships in the district has not been lost sight of.

The problem of mosquito-control in Soroti owes its difficulty mainly to three factors : the soil is of a sandy nature and shows little resistance to erosion ; storm-water, which may amount to as much as one inch an hour, cuts the surface in an almost inconceivable way, leaving in its track hundreds of pockets ready for the breeding of mosquitoes ; in many parts of the township the fall in land is so gradual that anything short of storm-water tends to stagnate. With the exception of two narrow ridges, Soroti was originally surrounded by swamps, all of which encroached on the township area, which has a diameter of three miles ; every dwelling was within one mile of one of these dangerous swamps.

As well as these major difficulties, mosquito-breeding in significant numbers took place in natural and man-made holes, wells, disused brick-pits, cattle hoof-prints and domestic collections of water.

1. *Measures Taken to Reduce Breeding*

The storm-water problem has been dealt with satisfactorily in certain parts by extensive clearing and levelling, and by building rubble drains which spread

the water out inside definite limits and thus retard its rate of flow and its cutting power. A typical rubble drain consists of : A square-section central channel 3 ft. wide ; a layer of broken granite 12 ft. wide, the centre of which lies over the granite filling the 3-ft. drain above described ; gently sloped earth banks, 15 ft. wide, on either side : these banks are planted with short grass, which is sufficient to prevent erosion by the evenly spread storm-water.

Three thousand yards of this type of drain have been put in during the last two years, and no repair has been necessary, while upkeep consists solely of occasional weeding and grass trimming.

There were 64,000 yards of earth drains in Soroti, which were all potential or actual breeding-places of *Anopheles*. A fair proportion of this total was superfluous, as many drains had been cut to help the anti-malarial planting and were no longer required when the eucalyptus trees had reached the age of three years. The work of filling in drains is shared by the Medical and Forests Departments ; about 8,000 yards have been so disposed of, and most of the remainder are regularly cleaned and oiled.

Anti-malarial planting with gums, cassia and later a type of African teak has changed most of the swamps inside the township from danger spots into productive areas. The last swamp so planted, however, was so deep and extensive that planting could not be undertaken until a straight earth drain had been cut to lead the water outside the township.

Numerous borrow-pits, brick-pits, and small holes have been filled in, and the hoof-print menace has been overcome by prohibiting the entry of cattle into the township. Redundant springs have been filled in and led into subsoil drains, and one has been reconstructed in the manner described in Part III, D, and rendered mosquito-proof.

A gang consisting of four labourers under the supervision of a sanitary orderly visits all potential breeding-places once a week and sprays them with an oily anti-malarial mixture. The use of Paris green is not feasible, as a suitable diluent cannot be obtained locally.

The Health Office keeps a record of the larvae collected from fixed control stations in various areas ; the specific identification of these larvae will eventually determine the value of the various plantations in the control of malaria.

2. *Measures Taken to Combat Adults*

The control of native dwellings in the township mentioned in Part III, A, should result in a diminution in the infectivity rate of *Anopheles*, and the gross adult mosquito population has already been considerably reduced by an extension of clearing and grass-cutting. All Government quarters and many of the better-class Indian residences are screened, and pyrethrum-spraying is encouraged ; it is hoped that such spraying on a large scale will shortly be undertaken by the Medical Department.

The mosquito-searchers collect adults from three areas in the township,

including European Government quarters, and weekly graphs are compiled from their results.

3. *Personal Prophylaxis*

The usual measures (mosquito-boots, mosquito-nets, etc.) not having proved efficacious by themselves, quinine prophylaxis was introduced about a year ago, when all European residents in Soroti and Serere were advised to take a daily dose of 5 gr. quinine. The malaria incidence, which used to average over three attacks a month among about 20 Europeans, has fallen to a negligible figure : during the last 10 months one European who had neglected to take quinine during an attack of dysentery contracted malaria ; the other case of malaria showed in the official returns was that of a visitor.

CONCLUSION

No mention has been made in the above account of the daily routine work of the various members of the European staff, but those who are familiar with medical administrative work will appreciate that the activities described are only a part of the day's work.

The first essential step for further progress is to have a subordinate staff so reliable and well-trained that constant supervision of the ordinary routine can be relaxed and time and thought given to new projects. It will thus be realized that important developments of a health programme depend partly on the assumption of some responsibility by the African himself, and that the best results will not ensue until he has grasped his duties as a citizen. Training of mind, character and body all play their part, and it is hoped that an organization such as that described will eventually act as a foundation on which a more efficient and elaborate health service can be built.

The authors are indebted to the Director of Medical Services, Uganda, for permission to publish this paper.

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EXPLANATION OF PLATE III

Fig. 1. Insanitary native hut, Teso. Compound not cleared. No out-buildings.

Fig. 2. Part of model compound, Teso. Hut, wash-place and latrine, from right to left.

Fig. 3. Usual type of native water-supply, Teso.

Fig. 4. Water-supply reconditioned.

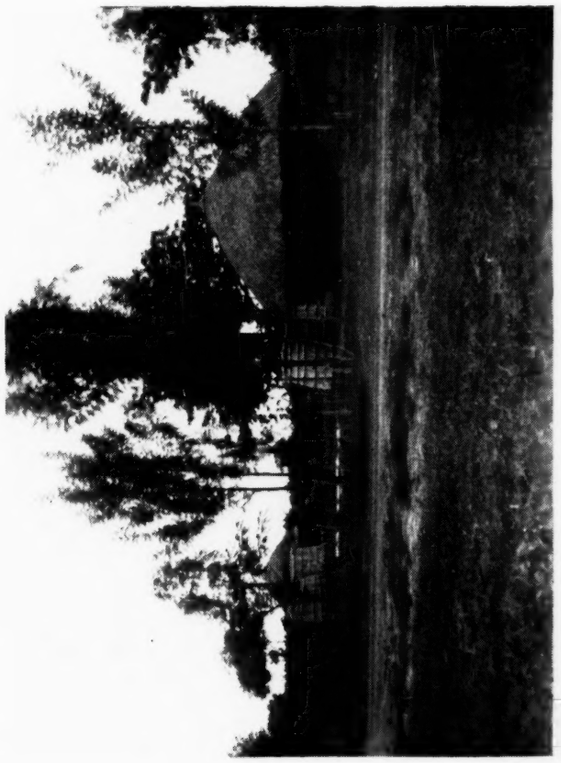


FIG. 2

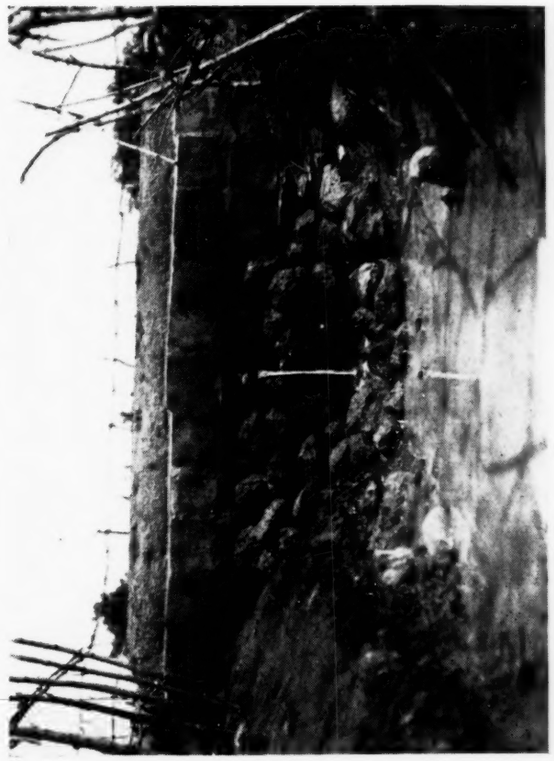


FIG. 4



FIG. 1



FIG. 3

H. R. Grubb, Ltd., Poplar Walk, Croydon.

ON PARTICULAR BINUCLEATE BODIES OFTEN FOUND IN HUMAN FAECES

BY

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(*Received for publication* 19 *December*, 1935)

When examining stained films of human faecal material in Java and in Holland, I have often come across particular binucleate bodies, the nature of which has puzzled me very much. Sometimes they were present in enormous numbers. For the sake of convenience, I have given them the name ' Bosman-cysts,' Private Bosman being the first patient in whose dejections I found them in great numbers.

The picture which they show is most characteristic in films stained with Delafield's haematoxylin or with Maier's haemalum ; it is less characteristic in those stained with Heidenhain's stain. Mostly the bodies are irregularly oval-shaped, sometimes more rounded ; the longest diameter in most specimens is about 5μ, but it may be as much as 8μ. They consist of an elliptical inner body and a wall. In almost every specimen the wall is wholly separated from the inner body by a clear ' empty ' space. The inner body shows a protoplasm and two intensively coloured dots (nuclei ?) that are somewhat variable in structure ; the protoplasm is not uniformily stained, but does not show apparent vacuoles or other sharply bordered inclusions. Specimens with one nucleus are very rare. In Heidenhain-stained specimens darker-coloured granules may sometimes be seen in the ' nuclei ' (figs. 5, 8).

The wall shows especially well in Delafield-coloured films (figs. 1–4). Except for its staining properties, it is characterized by irregular, sometimes very large thickenings and excrescences of its outer border. In Heidenhain-stained, well-decolourized specimens the wall is very pale, but its irregular outline can still be made out (figs. 5–12).

The structures described above are by no means rare : in Java I met with them frequently ; in Holland slightly less frequently. In the stools of some patients I found them on many successive days.

When studying *Dientamoeba fragilis* in cultures, it occurred to me that the Bosman-cysts might be the cysts of that protozoon. (In parenthesis it may be stated that amongst a group of asylum inmates *D. fragilis* proved to be the most frequently occurring intestinal protozoon ; probably it occurs in Java much oftener than I found it.) However, this hypothesis could not stand closer examination, and had to be discarded on the following grounds :—1. The Bosman-cysts never show nuclei similar to those of *D. fragilis* ; 2. In films from faecal material with many *D. fragilis*, Bosman-cysts were never met with ;

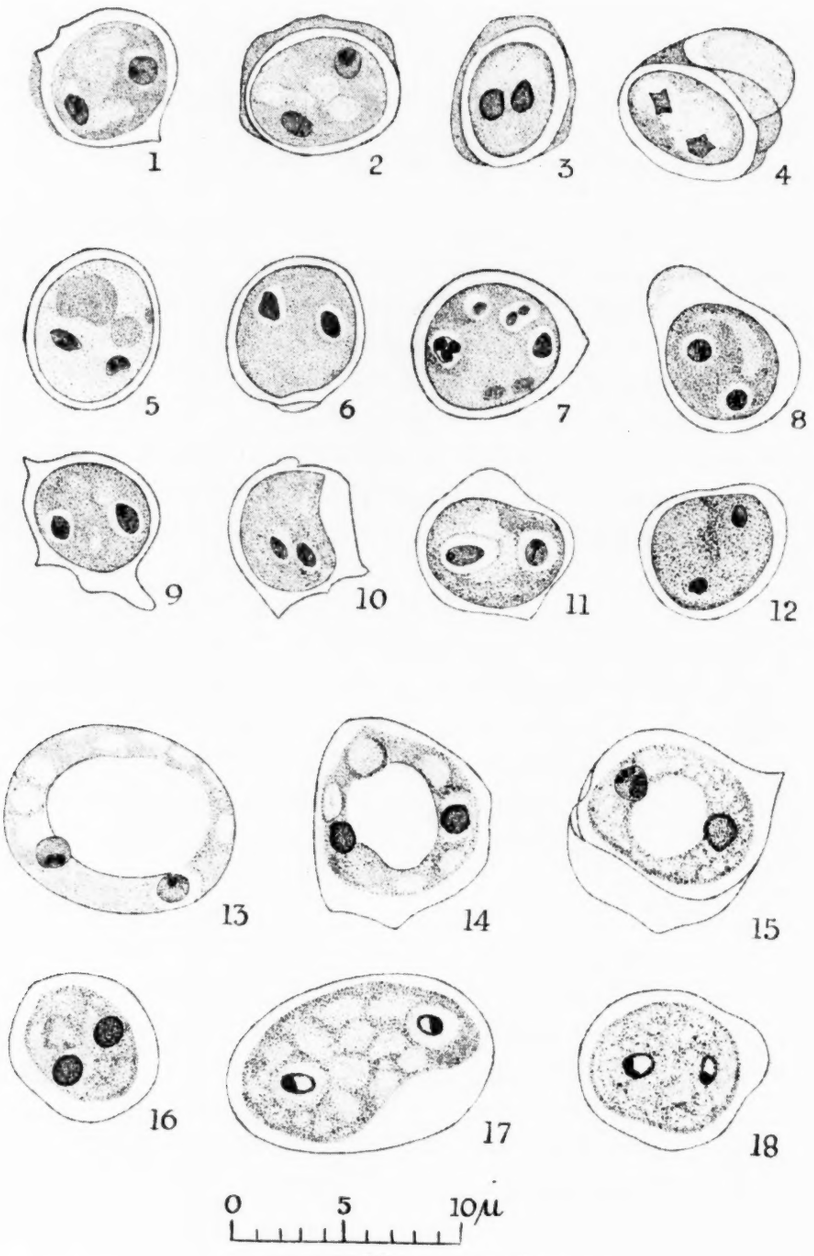


Fig. showing binucleate bodies often found in human faeces.

3. They were not seen in cultures of *D. fragilis*, some 40 strains of this amoeba being examined, and some of them being kept for months.

Figs. 13–18 represent structures from a case in which some specimens were found which suggested transitional forms between Bosman-cysts and blastocysts. In this film, stained with Maier's haemalum, almost all the blastocysts were binucleate and practically uniform in size and structure—more or less like that shown in fig. 13. It is difficult to say whether the specimen in fig. 14 is a blastocyst or a Bosman-cyst. It shows the ' loose ' wall of a Bosman-cyst and the large vacuole of a blastocyst. Fig. 15 very probably is a Bosman-cyst, but the occurrence of a rather large vacuole is exceptional. Figs. 16 and 18 are common Bosman-cysts ; fig. 17 is an unusually large one. It would, of course, be rash to conclude from the findings in this single slide that Bosman-cysts are a phase in the life-cycle of blastocysts. Nevertheless, the possibility of such an explanation must be borne in mind. At any rate, *if* the two are phases of the same species, this will be very difficult to prove ; simultaneous occurrence or successive occurrences of both in stools of the same person cannot serve in this case, as practically every human being is a carrier of blastocysts. In blastocystis cultures I never found Bosman-cysts.

It is curious that, while blastocysts have been widely studied, the structures described here have not yet attracted the attention of protozoologists occupied with intestinal protozoa. By beginners they may be confounded with small protozoal cysts.

Perhaps the solution of the problem of their real nature lies in the domain of the mycologist. Budding or other forms suggestive of multiplication stages were never met with.

ON THE POWER OF *GLOSSINA MORSITANS* AND *GLOSSINA PALPALIS* TO TRANSMIT THE TRYPANOSOMES OF THE *BRUCEI* GROUP

BY

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(*Received for publication* 27 *December*, 1935)

In a paper published in this journal in 1933, a comparison was made of the ability of *G. morsitans* and *G. palpalis* to transmit trypanosomes of the *brucei* group (Duke, 1933).

The conclusions reached were as follows :—

' 1. Evidence has been produced to show that *G. morsitans* is a more efficient vector of the trypanosomes of the polymorphic group than is *G. palpalis*.

' 2. Of a total of 3,119 *G. morsitans*, 203 (6·5 per cent.) were found on dissection to contain flagellates. Of a total of 3,236 *G. palpalis* treated in exactly the same manner, 95 (2·9 per cent.) contained flagellates. Of these grand totals, 881 flies of each species belonged to the experiments of Kleine and Fischer.

' 3. Although the carrying power of *G. morsitans*, as revealed by these experiments, appears to be almost twice as great as that of *G. palpalis*, even the former species, in the conditions of experiment prevailing at Entebbe, is but poorly adjusted to the requirements of the polymorphic group.

' 4. A strain of *T. gambiense*, judged to be non-transmissible by *G. palpalis* as the result of testing with several thousand flies, when tested by *G. morsitans* proved also non-transmissible by this species.'

During the last year, thanks to the co-operation of the administrative and medical authorities of Tanganyika Territory, a further supply of *G. morsitans* pupae has been obtained by air, and this investigation has been extended.

Details of the individual experiments are no longer necessary. Suffice it to say that paired boxes containing originally approximately equal numbers of flies were employed, and that the two boxes of each pair were fed on the same infected monkey at the same time and treated subsequently in exactly the same manner.

The infecting animals were monkeys, each infected with one of several strains of *T. gambiense* and *T. rhodesiense*, all of which were known to be transmissible by tsetse. Flies dying before the 10th day after the first infecting feed were disregarded.

The data were examined by Mr. J. S. McDonald, Laboratory Assistant to the Institute, and the results obtained were as follows :—

G. morsitans	*G. palpalis*
2,091 flies dissected.	2,854 flies dissected.
80 flies contained flagellates	70 flies contained flagellates
i.e. 3·83 per cent.	i.e. 2·45 per cent.

Examined by the formula $\chi^2 = \int\frac{(x-m)^2}{m}$, the value obtained for χ^2 is 7·75, the corresponding P value of which is much less than 0·01.

Differences as great as or greater than those observed in the series under investigation could occur by chance only once in considerably over 100 trials.

The values of χ^2 obtained in the earlier studies of this question were as follows :—

Series I	36·95	Series III	3·82
„ II	3·58	„ K and F	13·05

Series K and F was calculated from figures obtained by Kleine and Fischer in 1912–13 (Kleine and Fischer, 1913).

The additional evidence, therefore, confirms the conclusion drawn from the earlier investigations, namely, that *G. morsitans* is better able to transmit the trypanosomes of the *brucei* group than *G. palpalis*.

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A NOTE ON THE INFECTIVITY OF *TRYPANOSOMA RHODESIENSE* IN THE CROP OF *GLOSSINA PALPALIS*

BY

H. LYNDHURST DUKE

AND

KENNETH MELLANBY

(*Received for publication* 27 *December*, 1935)

In a paper on the development of *T. gambiense* in *G. palpalis*, Robertson (1913) writes: '. . . in some cases, part of the original blood may be retained in the crop unmixed, and apparently unaltered, for as much as 10 days or more, although frequent feeds have intervened. A case of this kind was also observed in an experiment conducted by Dr. Duke and Captain Fraser.'

In the routine experiments at the Entebbe laboratory, flies were given infecting feeds of mammalian blood on the second and fourth day after emergence, and were subsequently nourished on chickens. Trypanosomes together with mammalian red blood cells were not uncommonly found in the crops of flies dissected at least 14 days after their infecting feed. Such flies usually died with their crops distended. The trypanosomes appeared to be unchanged both in morphology and activity, and were perhaps slightly more numerous than they were originally in the animal's blood.

As flies of this type were available from time to time, it was decided to find out whether the trypanosomes in the crop were infective on inoculation into sensitive animals. Accordingly 3 inoculations of the crop-contents were made into white rats (red cells plus trypanosomes). Rat I received the crop-contents of one fly 12–14 days after the infecting meal; rat II the crop-contents of two flies, 10–12 days after the infecting meal; and rat III one fly 11–13 days after the infecting meal. None of these rats became infected.

During this brief sojourn in the fly at an average temperature of 24° C. the trypanosomes (*T. rhodesiense*) had therefore lost their infectivity.

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ON A NEW SPECIES OF NEMATODE, *AMPLICAECUM CACOPI* SP. NOV., FROM *CACOPUS SYSTOMA*

BY

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(*Received for publication* 6 *January*, 1936)

Eighteen females and six males were obtained from the stomach of *Cacopus systoma*. Worms of medium size. Body tapering towards the anterior extremity, posteriorly terminating abruptly in an obtuse cone at the end of a ventral coil. Mouth surrounded by three well-developed rectangular lips—one dorsal and two subventral, the former bearing two large papillae, and each of the latter bearing one large and one small papilla. All three lips bear on their inner margins the dentigerous ridges characteristic of the genus. Small interlabia are also present. Cuticle thick and smooth, marked with fine transverse striations and expanded in the posterior part of the male to form caudal alae. Only one large intestinal caecum present, broadest at its union with the intestine and narrowing anteriorly. Nerve ring very prominent, lying approximately at anterior fifth of the oesophagus. Excretory pore immediately behind nerve ring.

MALE. Length 11·4–18·6 mm.; maximum breadth 280–420μ. Length of oesophagus and intestinal caecum 1·9–2·5 mm. and 1–1·5 mm. respectively. Nerve ring from the anterior extremity of the body 370–600μ. Well-developed caudal alae present, into which project nine pairs of pedunculated papillae, of which five pairs are pre-anal and four pairs post-anal. Spicules short and equal, measuring 210–280μ. Accessory piece absent. Tail blunt, 230–300μ long, with a pointed button at the tip.

FEMALE. Length 14–34·5 mm.; maximum breadth 290–570μ. Length of oesophagus and intestinal caecum 1·96–3·5 mm. and 1·14–2·1 mm. respectively. Nerve ring from the anterior extremity of the body 380–730μ. Vulva approximately at the junction of the anterior and middle thirds of the body, 5–11·4 mm. from the anterior end and provided with two prominent lips—one anterior and the other posterior. Vagina long and muscular, running obliquely posterior in the body across the dorsal side of the body cavity. Uteri long and parallel, running posteriorly. Origin of ovarian coils a little anterior to the level of anus. Tail blunt, 210–350μ long, with a pointed button at the tip. Eggs large and oval, measuring 114–134 $\times$ 66–80μ, and provided with thin transparent shells.

Of the seven species of *Amplicaecum* described, the present form resembles *A. brumpti* Khalil 1926 in the number and arrangement of the caudal papillae, but differs from it in the presence of an additional pair of post-anal papillae, the great disparity in size, the relative position of the vulva and the presence of much shorter spicules.

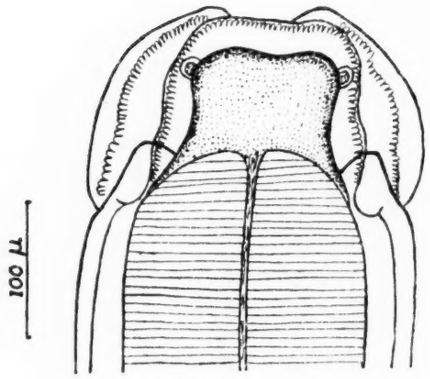


FIG. 1.

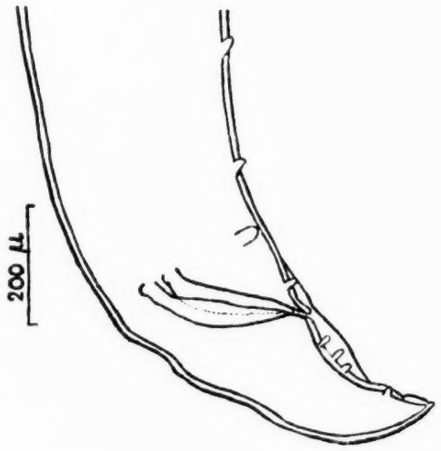


FIG. 2.

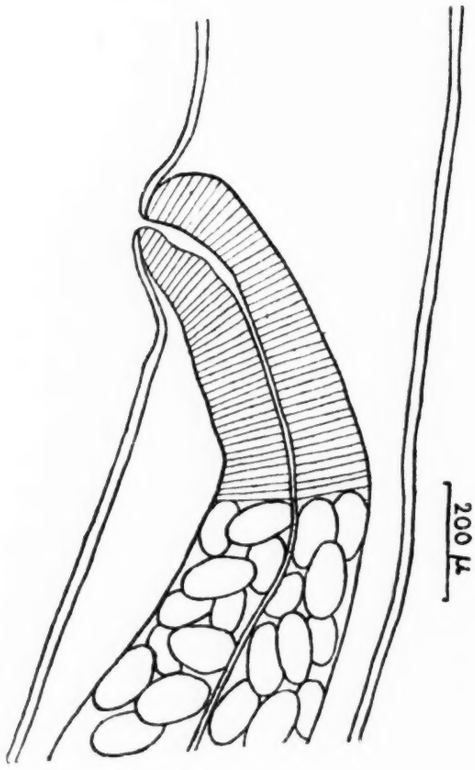


FIG. 3.

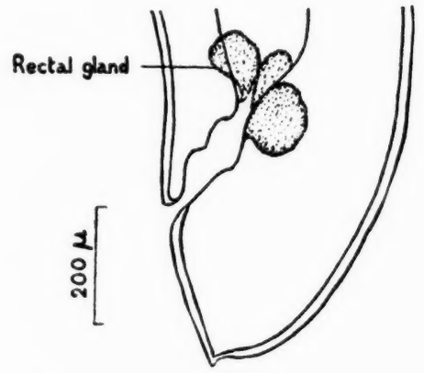


FIG. 4.

FIG. 1. *Amplicaecum cacopi* sp. nov. Head, dorsal view.

FIG. 2. Caudal end of male, lateral view.

FIG. 3. Vulval region, lateral view.

FIG. 4. Caudal end of female, lateral view.

TABLE

Species of *Amplicaecum*

	A. colurum Baylis 1919		*A. involuta* Gedoelst 1916		*A. varani* Baylis and Daubney 1922		*A. africanum* Taylor 1924	
	Male	Female	Male	Female	Male	Female	Male	Female
Length	Not known	46 mm.	12-13·5 mm.	20 mm.	22·2-24·9 mm.	24·75 mm.	22-30 mm.	32-50 mm.
Maximum breadth ...		1 mm.	43μ	59μ	730μ	800μ	460-680μ	0·66-1·2 mm.
Length of oesophagus ...		7 mm.	1·96-2 mm.	2·4 mm.	3·5-4·7 mm.		3-4·5 mm.	
Length of intestinal caecum		to 1·6 mm. from ant. end	$\frac{2}{5}$ oesophagus		0·9-1 mm.		3·57 mm.	
Length of spicules ...			930-950μ		500μ		850μ	
Caudal papillae—								
pre-anal (in pairs) ...			12 mm.		32 mm.		10-12 mm.	
post-anal (in pairs) ...			3 mm.		5 mm.		3 mm.	
Vulva from anterior end ...		17 mm.		8·5 mm.		6·5 mm.		Anterior to middle
Length of tail		230μ	240μ	240μ	160μ	320μ	310μ	300μ
Eggs		112-125 × 70-75μ		124 × 66μ		67·5-75 × 50μ		135 × 83μ
Host	*Lophoaëtus occipitalis*		*Camaeleon dilepis*		*Varanus salvator*		*Bufo regularis*	
Habitat			Stomach		Intestine		Stomach	
Locality	Uganda		Belgian Congo		India		West Africa	

	A. gedoelsti Yorke and Maplestone 1926		*A. brumpti* Khalil 1926		*A. novempapillatum* Sandground 1933		*A. cacopi* sp. nov.	
	Male	Female	Male	Female	Male	Female	Male	Female
Length	17 mm.	32-35 mm.	44 mm.	70 mm.	19-23 mm.	42-43 mm.	11·4-18·6 mm.	14-34·5 mm.
Maximum breadth ...	390μ	720 μ	500μ	750μ	600-700μ	1·2 mm.	280-420μ	290-570μ
Length of oesophagus ...	$\frac{1}{7}$ body length	$\frac{1}{8}$ body length	4·38 mm.	5·1 mm.	2·3-3·4 mm.	5·1-5·7 mm.	1·9-2·5 mm.	1·96-3·5 mm.
Length of intestinal caecum	$\frac{4}{5}$ oesophagus		2·3 mm.	2·7 mm.	1·6-2·4 mm.	4·1 mm.	1-1·5 mm.	1·14-2·1 mm.
Length of spicules ...	1·01-1·04 mm.		330μ		1·15 mm.		210-280μ	
Caudal papillae—								
pre-anal (in pairs) ...	10 mm.		5 mm.		9 mm.		5 mm.	
post-anal (in pairs) ...	3 mm.		3 mm.		3 mm.		4 mm.	
Vulva from anterior end ...		Anterior third		19 mm.		15 mm.		5-11·4 mm.
Length of tail	255μ	220μ	250μ	250μ	260-350μ	270μ	230-300μ	210-350μ
Eggs		120 × 60μ		110 × 64μ		125 × 85μ		114-134 × 66-80μ
Host	Toad		*Rana esculenta*		*Astylosternus robustus*		*Cacopus systoma*	
Habitat	Stomach		Stomach and intestine				Stomach	
Locality	Belgian Congo		Corsica		U.S.A.		Burma	

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THE CHARACTERS OF *ANOPHELES MARSHALLI* THEOBALD AND *A. MARSHALLI* var. *PITCHFORDI* GILES IN THE ADULT, LARVAL AND PUPAL STAGES

BY

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AND

H. S. LEESON

(*Received for publication* 6 *February*, 1936)

It is only too well known that the exact determination of specimens resembling *A. marshalli* Theo. and *A. marshalli* var. *pitchfordi* Giles is often a matter of extreme difficulty or even impossibility. This is partly due to confusion with other forms now recognized as distinct species because of marked differences in the morphological characters and early stages which have been described by Ingram and De Meillon (1929), De Meillon (1928, 1931*a*, 1931*b*) and Evans (1929, 1931). The key on p. 54 gives a means of separating *A. marshalli* from these forms. The difficulty is also due to the fact that the limits of the type form of *A. marshalli* and var. *pitchfordi* were unknown, and that many specimens encountered are somewhat intermediate in character between these two forms as defined, from examination of the type specimens, by Edwards (1912), Christophers (1924) and Evans (1927).

From the studies by one of us (B. De M.) of material bred from isolations in the Transvaal and of var. *pitchfordi* from the type locality, it is now possible to define more clearly the limits of these forms as they occur in South Africa. Material of *A. marshalli* collected by one of us (H. S. L.) from the type locality is found to agree with the Transvaal material ; and we are, therefore, able to give a comprehensive account of the type form of this species as it occurs in South Africa, and of var. *pitchfordi* which is evidently a local variety occurring in the neighbourhood of Eshowe, Zululand.

The investigations of one of us (B. De M.) have shown that the costal markings are very variable in the type form and may approach the condition characteristic of var. *pitchfordi* (see figs. 1, C; 4). In this connection it is interesting to record that in a specimen among a collection made by one of us (H. S. L.) the wings are markedly dissimilar, one having costal markings as in the type of *marshalli*, the other approaching that of var. *pitchfordi* in this respect. Further, examination of this collection shows that the mesonotal scales may be very narrow on the posterior half, so that this character is of no value for separating var. *pitchfordi* from the type form of *marshalli*. It is now evident that many specimens that would formerly have been identified as the variety will now be found referable to the type form.

The form of *marshalli* investigated by Gibbins (1932, 1933*a*) in Uganda has been found to be distinct in larval and pupal characters from the type form, and was recently described as a new variety, *A. marshalli* var. *gibbinsi* Evans (1935). It will be possible to determine the status of the *marshalli*-like forms occurring elsewhere in East Africa when the characters of the early stages are known.

Anopheles (Myzomyia) marshalli Theobald type form

The following are the characters of the type form of *A. marshalli* as it occurs in South Africa :—

A small to medium-sized species. ♀ *palpi* with 3 pale bands, the 2 distal ones very broad. *Mesonotum* with narrow curved or very narrow scales, sometimes narrower posteriorly. *Wings* commonly with subcostal and subapical pale spots small, but sometimes larger with their combined length approaching that of the third and fourth dark spots together ; base of costa with 1 or 2 pale interruptions, which may be minute. First vein with third main dark area interrupted by a pale spot, which may be very small. Third vein usually mainly pale ; other veins extremely variable. Legs unspeckled. Hind tarsi with pale apical rings on first 4 segments, the rings sometimes very narrow.

ADULT. ♀. *Head* (fig. 1, D). Pale scales white ; frontal tuft well developed. Antennae with pale scales on about the first 2 or 3 flagellar segments. Palpal index about 0·3–0·4. Palpi with a narrow pale band at the apex of the second segment, a broad pale band involving the apex of the third and base of fourth segments, and another about equally broad occupying the whole of the last segment and apex of the fourth. The intervening dark band may be much shorter than the subapical pale band, or it may be equal to or very slightly longer than this band.

Pharynx. The structure in 2 ♀♀ examined from Southern Rhodesia and 1 from the Transvaal is very similar to that in *A. demeilloni* (Evans and De Meillon, 1933). About 12 or 13 well-developed rods and cones.

Thorax. Mesonotum with the usual markings. Median grey area clothed with pale scales which are usually greyish-white or white and vary considerably in width and length. Scales sometimes well developed over the whole area ; in other cases, well-developed scales may be largely confined to the anterior half or less, the posterior portion bearing mostly very narrow, almost hair-like scales (fig. 1, J). There is considerable variation in the width of the well-developed scales (fig. 1, E–H), the broadest type being rather broadly narrow-curved (index about 8·5). A few long narrow scales may be intermixed with the bristles above and in front of the wing root.

Abdomen. No scales present, even on cerci.

Legs (fig. 1, K). Knee spots distinct on tibiae, though sometimes small. Tarsi of front and mid legs usually with pale apical rings on the first 2 or 3 segments, the rings variable in width and distinctness, but usually not very

pronounced. First 4 segments of hind tarsi with apical pale rings which are commonly rather small, being only about as long as the apical width of the segments and sometimes much less than this.

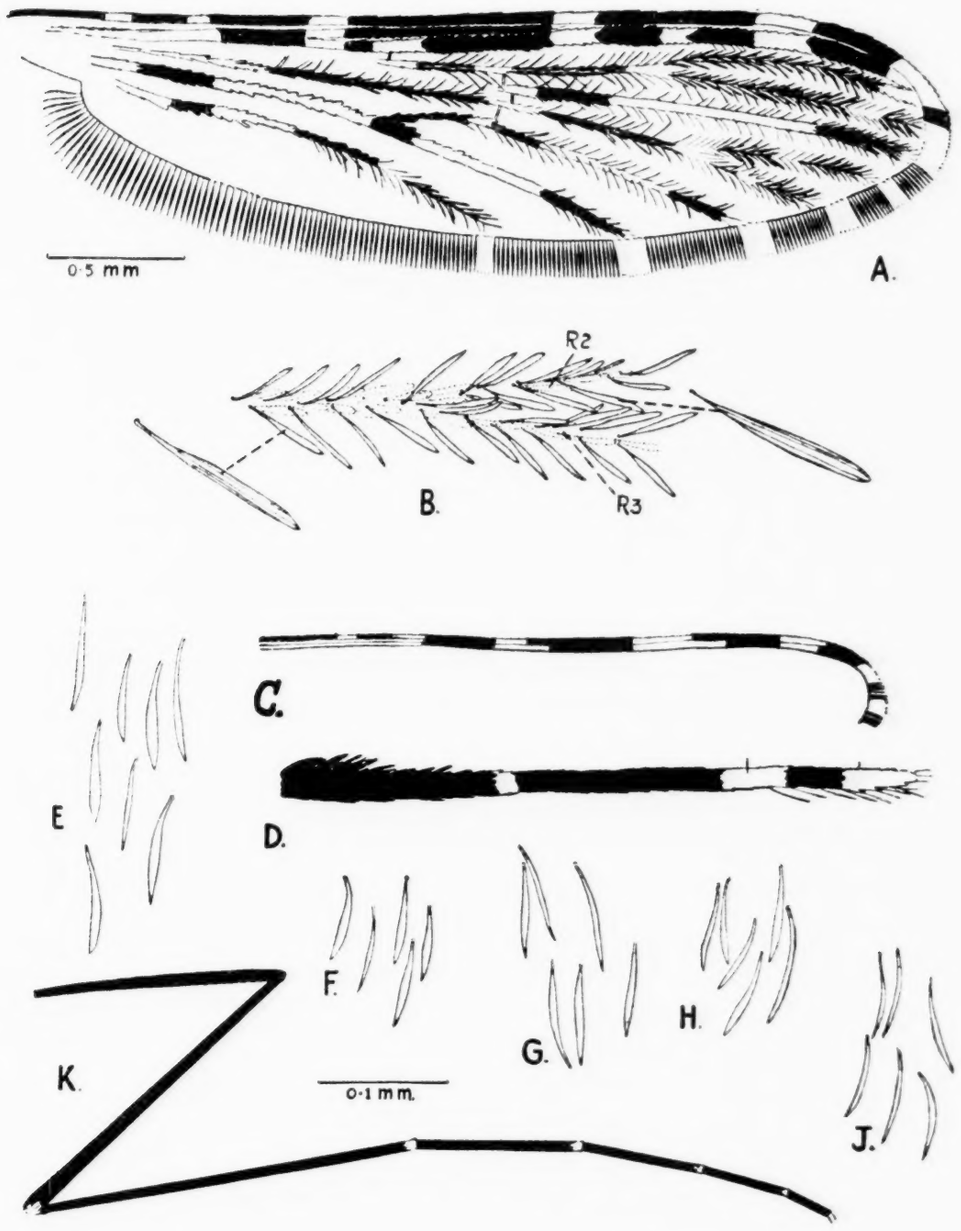


FIG. 1. *Anopheles marshalli* Theo. type form. A.—Wing with dark type of costa; B.—Scales from second vein; C.—Costal border of wing, with maximum extent of pale areas on outer half; D.—♀ palp with typical banding; E.–H.—Scales from anterior half of mesonotum of different specimens, showing variation in size and shape; J.—Scales from posterior half of specimen from which H. was drawn; K.—Hind leg; R_2 and R_3.—Branches of second vein. (C. from drawing by B. De Meillon; the rest by A. M. Evans.)

Wings (fig. 1, A, C). Pale scales creamy-white to yellow. Costa with the 4 main pale spots normally present, but the sector spot occasionally greatly reduced ; base of costa with one or two pale interruptions which may be very minute. Pale spots on outer half of costa very variable in length, commonly short, as shown in fig. 1, A, but one or more may be considerably longer, the maximum amount of pale scaling observed being about as in fig. 1, C. First vein similarly marked but with basal portion pale, accessory sector spot present and a pale interruption in the third main dark area. Second, fourth and sixth veins extremely variable, sometimes mainly pale, in other cases dark with pale spots greatly reduced. Third vein with a large median pale area and dark spots or areas towards the ends variable in size. Fifth vein with the usual 6 dark areas very variable in size, dark spot present on lower branch at fork, though sometimes minute. Fringe with dark spot at apex of wing and with pale spots at ends of third to fifth veins ; pale spot also present, though sometimes indistinct at end of sixth vein. Basal portion of fringe sometimes with a pale spot, which may be confined to the border scales. Outstanding scales of veins long and narrow (fig. 1, B).

Wing length. About 3·0–3·5 mm.

ADULT. ♂. *Palpi.* Club with the 3 pale areas either moderately large or relatively small, that at its base sometimes reduced to 1 or 2 pale scales. Stem without pale scales in specimens examined.

Terminalia (fig. 2). The structure in three specimens from Southern Rhodesia was as follows. Parabasal spines 5 in number, including the long slender accessory. Harpagones with club and apical bristle about equal in length; a single outer accessory hair from rather more than 1/2 to 3/5 length of apical ; no inner accessory hair ; microtrichia of inner wall relatively long and dense. Phallosome with about 4 or 5 pairs of leaflets, the longest about 2/5 to nearly 1/2 its length.

PUPA. The following are the principal characters of Transvaal material bred out by one of us (B. De M.) :—

Paddle. External border with fringe of spines on about the distal half, becoming gradually longer and more slender and replaced by rather long hairs (hair-like processes) ; fringe continued beyond the apical bristle along the distal border as very delicate, short, sparse hairs invisible in some mounts. Apical bristle bent and variable in length, but at least 1/4 the length of the paddle ; subapical hair with about 4–6 branches.

Spine A. VIII, with about 8–10 branches ; IV-VII, finely pointed; VII, about 2/5 length of segment or more ; V and VI, somewhat shorter than that on VII ; IV, variable, commonly about half that on VII or slightly more, but sometimes much shorter (only 2/9 in one specimen) ; III, very short ; II, minute, unchitinized.

Bristle B. V-VII, from about 5/9 to rather more than 2/3 length of segments, branched. Number of branches about as follows : VII, 5–6 ; VI, 6–9 ; V, 7–9 ; IV, 7–11.

Bristle C. V-VII, simple and about equal in length to segments ; IV, about 4–8 branches ; III, about 6–9.

Hair C. VI, bifid.

Hair 4. Branches about as follows : VII, 5 ; V and VI, 5–7 ; IV, 4–8 ; III, 7–8.

Respiratory trumpet. Meatus about 0·2–0·26 length of trumpet.

The pupa described by Senevet (1931) differs from Tzaneen material in certain respects, notably in the very short paddle bristle (1/7 the length of the paddle). It is probably that of another form.

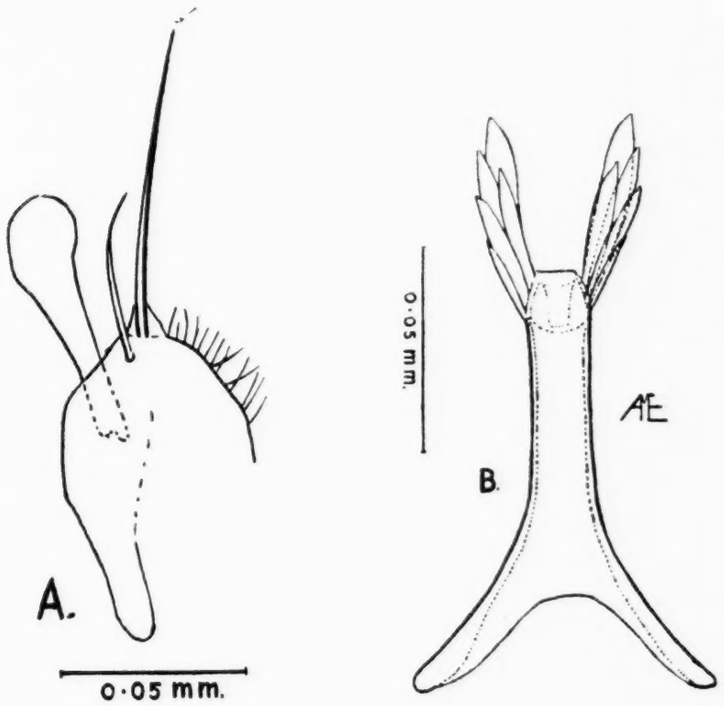


FIG. 2. *Anopheles marshalli* type form. ♂ terminalia. A.—Harpago ; B.—Phallosome.

The great variation in the relative length of spine A on IV is a very unusual character.

LARVA (fig. 3). The larva of *A. marshalli* has been described by Ingram and De Meillon (1929) and by De Meillon (1928, 1931*a*, 1931*b*) from Transvaal material. Very recently the characters have been still further elucidated by one of us (B. De M.) from a large number of isolations. Larvae taken in Southern Rhodesia by one of us (H.S.L.), an isolation being made, have been found to agree with Transvaal material, and specimens showing the striking peculiarity in the outer and posterior clypeal hairs have been taken near the type locality.

The most important diagnostic characters are illustrated in fig. 3. *Inner clypeal hairs* simple and of moderate length, outer usually simple but occasionally

with 2 or 3 branches, not much more than half length of inner ; posterior usually simple but occasionally branched or even feathered. *Antennae* with a group of elongated spines on basal half of inner border. Long *mesopleural bristles* both simple. Typical abdominal *palmate hairs* with filament variable in length and shape but tending to be relatively short. Saddle hair branched (usually at least 5). *Tergal plates* of moderate size.

A medium-sized larva. General colouration dark, partly owing to tergal plates.

Head (fig. 3, A—C). *Inner clypeal hairs* simple (or occasionally with a barb), of moderate length (about 1/3–3/8 length of fronto-clypeus) and, as usual, finely drawn out distally ; *outer* usually simple, rather more than half length of inner ; *posterior* hair simple or branched, sometimes with 5–9 lateral branches ; length varying from 1/2 to more than 2/3 that of inner hairs. *Post frontal hairs* simple (normally, at least). *Antennae* rather stout ; basal half of inner border with a group of relatively long spicules (in some cases, at least), shaft hair of small to moderate size, and situated at about 1/6 to 1/4 of the distance from the base ; apical hair divided into about 6–8 branches.

Thorax (fig. 3, D—F). *Shoulder hairs.* Chitinized bases of inner and middle hairs either joined or separate, and, when separate, they may be closely approximated or, as one of us (B. De M.) has recently discovered, very far apart, almost but not quite as in a *Neomyzomyia*. *Palmate hair* with about 10–13 lanceolate leaflets of normal length as indicated in the figures, apices finely pointed or blunt. *Pleural bristles* as in other species of subgroup I of *Myzomyia* ; *mesothoracic posterior ventral* with about 2–5 branches ; basal spines small.

Abdomen (fig. 3, G—N). *Palmate hairs.* I, not fully developed but with about 11–13 leaflets with serrated shoulder and differentiated filament. II to VII, fully developed. IV and V, leaflets about 0·85–0·11 mm., sometimes broad, but variable in width ; serrations usually sharply pointed ; filament very variable in shape, sometimes tapering, with linear termination as shown in fig., but in other larvae very short and abruptly pointed, the shape approaching that in var. *pitchfordi* (fig. 5). *Lateral hair* on IV and V with about 6–8 branches. *Saddle hair* relatively short and much branched, about 5–11 (commonly 7 or 8). *Inner caudal hair* with the longer branches hooked at tip. *Tergal plates* of moderate size, that on V about 3/5 the distance between palmate hairs or rather more. *Median plate* of *spiracular scoop* with or without distinct lateral arms. *Pecten* with about 5 long teeth separated by about 3 or 4 groups of short teeth. Short teeth usually rather small, the longest of them not being much more than half the length of the ventral long tooth. Teeth with numerous, rather fine spicules.

Variation. One of us (B. De M.) finds that the type of palmate hair with very short, abruptly pointed filament (much as in *pitchfordi*, fig. 5, B) occurs commonly among larvae from Komatipoort and Tomango. The material described in the memoir (De Meillon, 1931*b*) was from this district. The

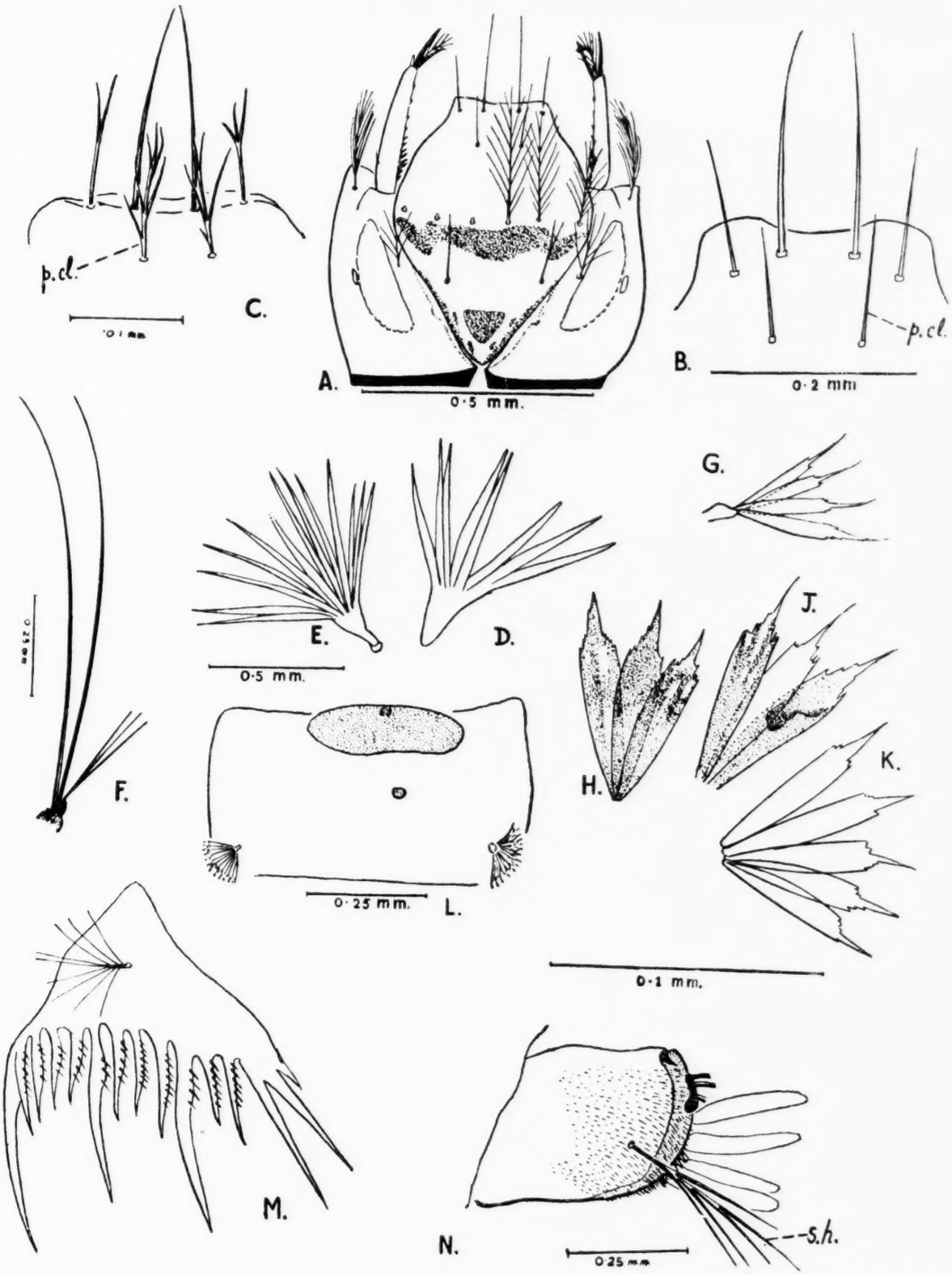


FIG. 3. *Anopheles marshalli* type form. Larval details. A.—Head, dorsal aspect (shaft hair omitted from right antenna); B.—Clypeal hairs, typical; C.—Clypeal hairs, posterior atypical; D.-E.—Thoracic palmate hairs from different larvae; F.—Mesopleural bristles; G.—Leaflets of palmate hair of abdominal segment I; H.-J.—Leaflets from IV, showing variation in length of filament; K.—Leaflets from III; L.—Outline of segment V to show tergal plate; M.—Pecten, typical arrangement; N.—Anal segment to show saddle hair. *p.cl.*—Posterior clypeal; *s.h.*—Saddle hair. (C., F. and N. by H. S. Leeson; A. composite, mainly by H. S. L.; D. and K. by B. De Meillon; the rest by A. M. Evans.)

peculiar variations exhibited in the characters of the posterior clypeal hairs and the shoulder hairs are most unusual in a species of group *Myzomyia*.

DISTRIBUTION IN SOUTH AFRICA. Southern Rhodesia, Transvaal, Natal, Zululand.

The type form probably extends well into East and Central Africa, and a ♀ agreeing with this form is part of a small collection taken in Southern Nigeria in 1900 by Drs. Annett, Dutton and Elliott. This specimen is in the collections of the Liverpool School and was at first labelled '*P. costalis* ?' From what is now known about the larval characters, however, it is obvious that material taken north of Southern Rhodesia cannot definitely be referred to this form until the larval characters are compared with those of *A. marshalli* and its varieties *pitchfordi* and *gibbinsi*. It is possible that undescribed larval varieties may occur in parts of the region.

Anopheles marshalli var. **pitchfordi** Giles

Anopheles pitchfordi Giles, Rec. Anoph. p. 34 (1904)

One of us (B. De M.) has recently reared from isolated larvae taken in or near the type locality 30 specimens agreeing closely with the type ♀ but having the mesonotal scales of uniform width and exactly as in certain specimens of *marshalli* from the Transvaal. Examination of Southern Rhodesian material, taken by one of us (H.S.L.), however, shows that there is a good deal of variation in this respect in the type form, and it is now obvious that the type of var. '*pitchfordi*' with the scales on the posterior 2/5 of the mesonotum hair-like and yellowish is an individual variation from the normal condition in this variety.

The following characters are found by one of us (B. De M.) to distinguish var. *pitchfordi* from the type form :—

ADULT. ♀. *Legs*. Fore-tarsal banding more pronounced. Dr. Edwards has kindly examined the type and finds that this character is present.

Wings (fig. 4). Costa with pale spots on outer half very extensive, combined length of subcostal and subapical pale spots usually greater than that of third and fourth dark spots, but sometimes slightly less. There is a considerable amount of variation in the size of the outer costal spots and an extreme case is illustrated (fig. 4, B). Basal quarter of costa sometimes entirely pale. Subcosta and first vein characteristically with long uninterrupted pale areas. The apex of the wing with or without a dark spot between the ends of the branches of the second vein.

Pharynx. In a limited amount of material the number of cones varied from 10–12, but further investigation is necessary before it can be stated that this represents a distinction from the type form.

Variation. The length of the pale palpal bands in the ♀ was found to be variable, a specimen occurring in which the intervening dark band was only 1/4 the length of either pale band.

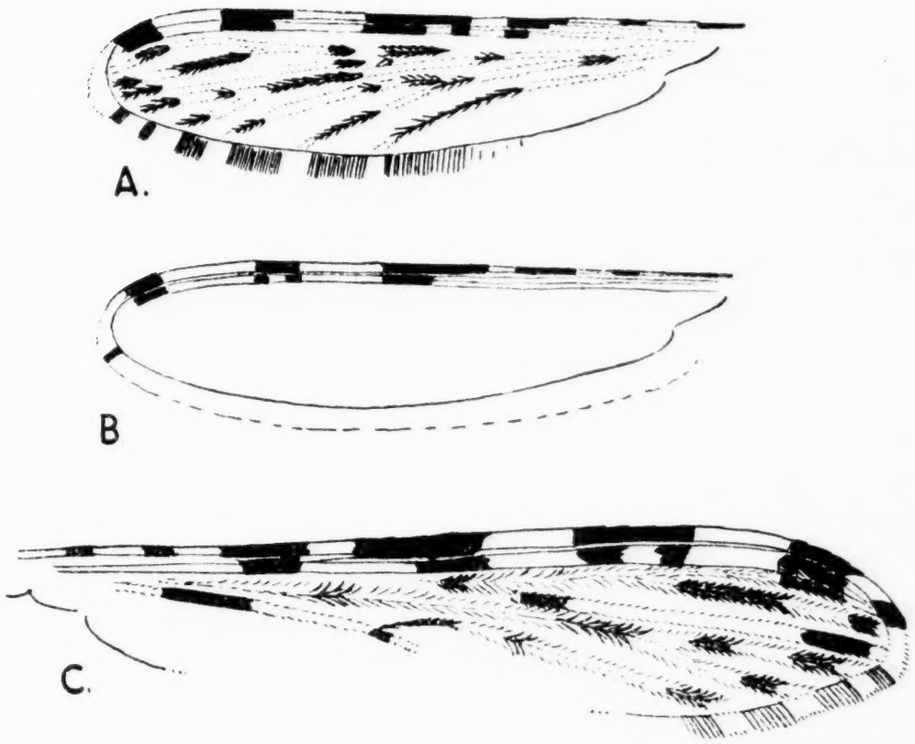


FIG. 4. *Anopheles marshalli* var. *pitchfordi* Giles. Wings of specimens from type locality. A.—Wing showing normal extent of pale areas on costa and wing field ; B.—Costal border showing extreme extension of pale areas ; C.—Part of wing of type ♀, to larger scale. (A. and B. by B. De Meillon ; C. by A. M. Evans.)

PUPA. No constant differences from that of the type form could be found, but the average length of the apical paddle bristle appears to be greater than in the type form.

LARVA (fig. 5). The following characters are found to distinguish var. *pitchfordi* from *A. marshalli* type form :—

Thoracic palmate hair much smaller than in type form (cf. figs. 3, D ; 5, A). Owing to its small size the hair is very difficult to see in living or uncleared larvae.

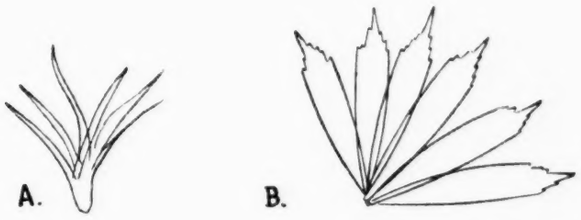


FIG. 5. *A. marshalli* var. *pitchfordi*. Larval details. A.—Thoracic palmate hair ; B.—Palmate hair of III. (A. to same scale as fig. 3, E. ; B. as fig. 3, H.–K.). (Drawn by B. De Meillon.)

Typical abdominal palmate hairs. Leaflets with very short, often stumpy filaments (fig. 5, B) which distinguish the larvae from those of the type form (except examples from Komatipoort and Tomango).

DISTRIBUTION. Zululand, Eshowe district.

Specimens recorded from the Belgian Congo, Dar-es-Salaam, Uganda and other regions cannot be definitely referred to this variety until the larval characters have been compared with those of Zululand material.

PROVISIONAL KEY FOR DISTINGUISHING *Anopheles marshalli* FROM ITS VARIETIES AND FROM OTHER ETHIOPIAN SPECIES OF GROUP *Myzomyia* WITH TWO BROAD PALE PALPAL BANDS IN THE ♀ AND WITHOUT A CONTINUOUS PALE AREA ON THE HIND TARSI

1.	Third dark area on first vein always without pale interruption	*A. keniensis* and allied forms, *A. freetownensis*, *A. moucheti*, *A. domicolus*, *A. flavicosta*, *A. barberellus* or atypical *demeilloni*.
	Third dark area on first vein with pale interruption (normally, at least)	2
2.	Mesonotal scales mainly rather broad...	*A. hargreavesi*, *A. austeni*.
	Mesonotal scales narrower, narrow-curved to almost hair-like	3
3.	Pale areas on outer half of costa much longer than succeeding dark areas (so far only identified from Eshowe District, Zululand)	*A. marshalli* var. *pitchfordi*.
	Pale areas on outer half of costa much shorter than succeeding dark areas	4
	Pale areas on outer half of costa not markedly different in length from succeeding dark areas	6
4.	South Africa	*A. marshalli* type form.
	Elsewhere in Africa	5
5.	Larva with inner clypeal hairs very short (not more than about ¼ length of fronto-clypeus) and rather abruptly pointed, appearing stumpy	*A. marshalli* var. *gibbinsi*.
	Larva with inner clypeal hairs of normal length (about ⅓ length of fronto-clypeus or more) and finely tapering	*A. marshalli* type form.*
6.	Larva with thoracic palmate hair very small (so far only identified from Zululand)	*A. marshalli* var. *pitchfordi*.
	Larva with thoracic palmate hair of normal size ...	*A. marshalli* type form.†

* Or perhaps undescribed larval variety.

† Or perhaps larval variety.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE GENERA OF THE FAMILY MUSCIDAE TESTACEAE ROBINEAU-DESVOIDY BASED ON A COMPARATIVE STUDY OF THE MALE AND FEMALE TERMINALIA

THE GENUS CORDYLOBIA GRÜNBERG (sens. lat.)

(*Continued from Vol.* 29, *page* 230)

BY

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(*Received for publication* 10 *February*, 1936)

The genus *Cordylobia* was erected by Grünberg (1903) for the well-known African warble fly, *anthropophaga* Blanchard. Surcouf (1920) in his revision placed the genus in the family Muscidae Testaceae in his group 4, allying it among others with the genera *Bengalia* and *Auchmeromyia*. As the life-history of *anthropophaga* is very well known, there is no necessity for me to refer to it here, beyond mentioning that the larva is a dermal parasite and is adapted not only for entering the unbroken skin of the host, but for remaining in it when entry has been effected. Surcouf recognizes two other species of *Cordylobia*, one of which, *rodhaini* Gedoelst, he has removed to his new genus *Stasisia* specially erected for it. The third species, *praegrandis* Austen, it will be remembered, lives in the burrows of the Aard Vark, and though the larva is not known it is believed to be a blood-sucker. I consider that this species is a true *Auchmeromyia*, for the reasons given in my paper (1935) on that genus. There are two other warble flies whose larvae live in the skin of animals and which, as will be shown in this paper, also belong to the genus *Cordylobia*. One of these was described by the late Dr. Aldrich (1923) under the name *Booponus intonsus*; its larval stages and life-history have been described by Woodworth and Ashcraft (1923). The larva lives in the skin of the foot of the carabao in the Philippine Islands, causing considerable lameness. The other species has been described by Austen (1930, 1932), who placed it in the genus *Elephantoloemus*, naming it *indicus*. The larva of this small fly lives in the skin of the Indian elephant, and as far as is known at present is only found in Burma. Although I have been able to study the larva of *B. intonsus*, I have unfortunately not had an opportunity of examining the ♂ and ♀ terminalia. At present only the ♀ fly is known. Through the kindness of Mr. J. Smith, M.R.C.V.S., I have been able to study not only the larva but also the ♂ and ♀ terminalia of *E. indicus*. I have not been able to study the ♂ and ♀ terminalia of *Stasisia rodhaini*, but fortunately the ♂ terminalia have been illustrated by Rodhain and Bequaert (1916). Through the kindness of Dr. Lamborn, Mr. Cuthbertson

and Mr. Potts, I have been able to study the ♂ and ♀ terminalia of *Neocordylobia roubaudi*, a species recently described by Villeneuve de Janti (1929). I wish to take this opportunity of thanking these gentlemen for the specimens, and Dr. Villeneuve de Janti for kindly determining his species. I propose in this paper to note and illustrate the important diagnostic characters of the ♂ and ♀ terminalia of *anthropophaga* Grünberg, *indica* Austen and *roubaudi* Villeneuve. As the segmentation of the abdomen is as in other muscids, I do not intend to refer to it.

Cordylobia anthropophaga Blanchard. SALIENT DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. The male terminalia are small, yellow and inconspicuous.

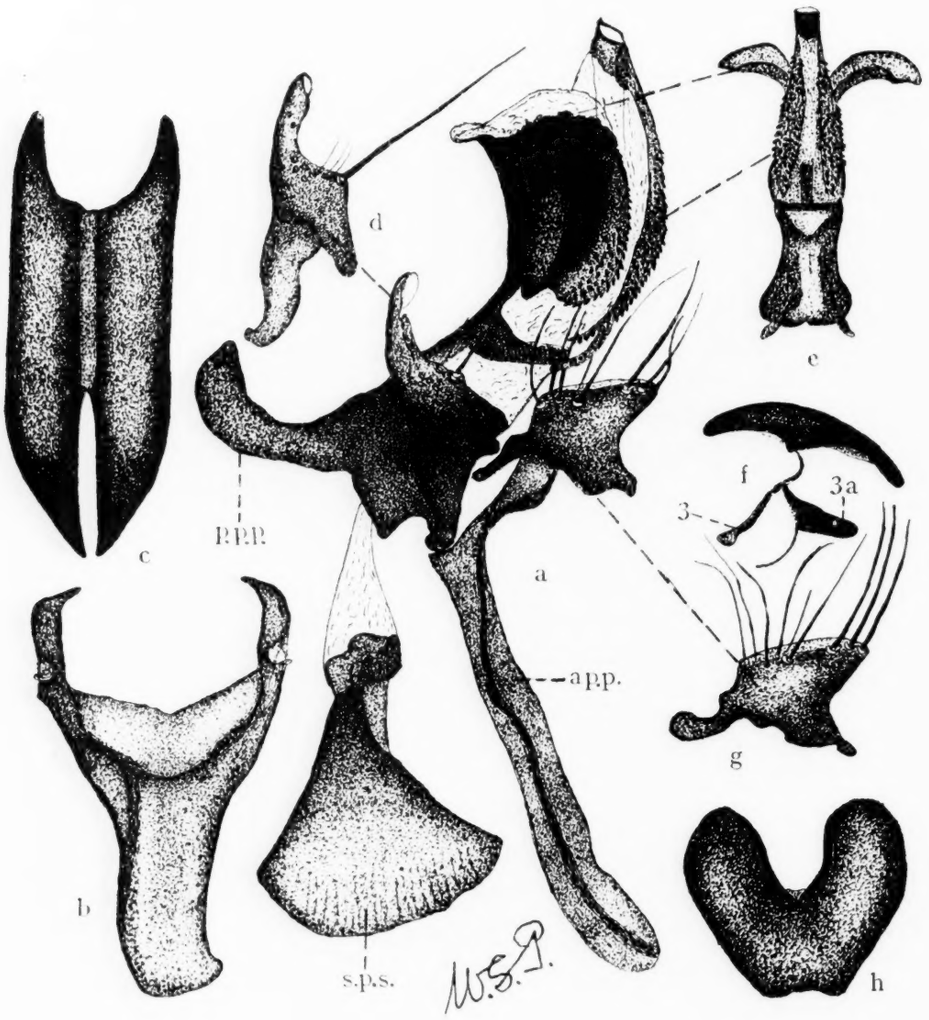


FIG. 1. *a.*—Phallosome, sperm pump sclerite (*s.p.s.*), apodeme (*ap.p.*), posterior process of phallosome (*p.p.p.*) and one paramere of *anthropophaga* in side view ; *b.*—Ninth tergo-sternum ; *c.*—Ventral view of anal cerci ; *d.*—Posterior part of right paramere ; *e.*—Dorsal view of phallosome ; *f.*—Lateral view of anal cercus and ninth coxite (3, proximal ; *3a*, distal segment) ; *g.*—Anterior part of right paramere ; *h.*—Fifth sternum.

Sternum 5 is large, with a V-shaped incision, as in fig. 1, *h*. *Ninth Tergo-Sternum.* As in fig. 1, *b*. *Ninth Coxite. Distal Segment.* Lateral view. Fig. 1, *f*. Small, with bluntly rounded end turned inwards and forming a lateral clasper ; the proximal segment is a stout rod articulating in the usual way with the posterior process of the ninth tergo-sternum. *Anal Cercus.* Ventral view. Fig. 1, *c*. Broad, ending in a short dorsally directed point, slightly less than anterior half free, joined to its fellow ; lateral view as in fig. 1, *f*, the pointed end appearing in this view blunt. *Phallosome.* Lateral view. Fig. 1, *a*. Short and broad, ventral wall sloping backwards to become continuous with stout posterior process; the proximal part is a short wide chitinous tube, membrane joining it to the distal portion, as is usual. The distal portion consists of a wide ventral part which is expanded and projects laterally, wing-like (fig. 1, *e*), and is armed with numerous short backwardly directed spines ; this part is joined by a bar basally and by membrane distally to the narrower dorsal portion ; the bar of chitin extends for a short distance in the middle line (median shaft) towards the distal end (fig. 1, *e*) ; on each side the membrane is strengthened by a strong chitinous spined flap ; it ends distally in the short wide opening of the ejaculatory duct, which is surrounded by a chitinous collar (fig. 1, *a*, *e*). *Paramere.* Lateral view. *Anterior Part.* Fig. 1, *a*, *g*. The anterior part of the paramere is a short sloping plate armed with 8–10 rather long hairs. *Posterior Part.* Fig. 1, *a*, *d*. The posterior part is an upstanding plate with a long ledge bearing one very long hair and several shorter ones in addition to the usual small sensory spines or hairs.

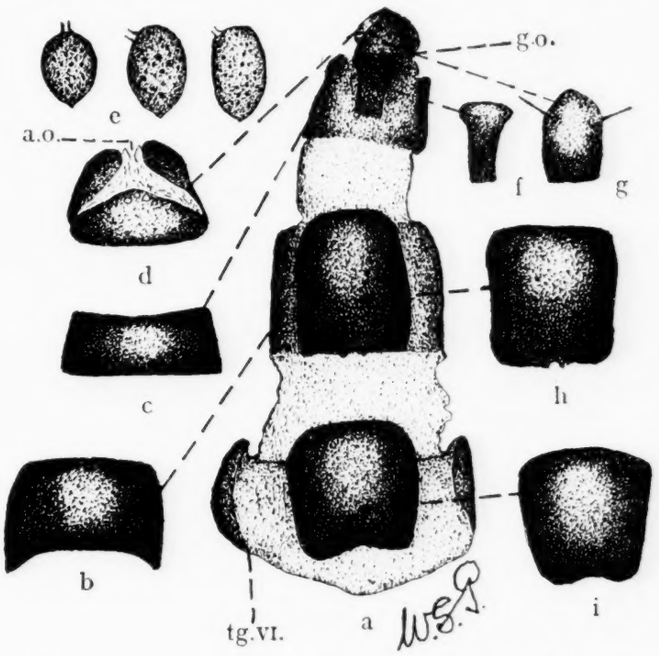


Fig. 2. *a*.—Ventral view of extended ovipositor of *anthropophaga* showing genital opening (*g.o.*) and sclerites (tergum six, *tg.vi.*) ; *b*.—Seventh tergum ; *c*.—Ninth tergum ; *d*.—Tenth tergum and anal cerci (anal opening, *a.o.*) ; *e*.—Spermathecae ; *f*.—Ninth sternum ; *g*.—Tenth sternum ; *h*.—Seventh sternum ; *i*.—Sixth sternum.

♀. The ♀ terminalia are illustrated in fig. 2. The ovipositor is relatively long, and all the sclerites are well represented, especially the sterna, which are stout plates. The spermathecae are illustrated in fig. 2, *e*. As these illustrations speak for themselves, it is unnecessary to describe the sclerites.

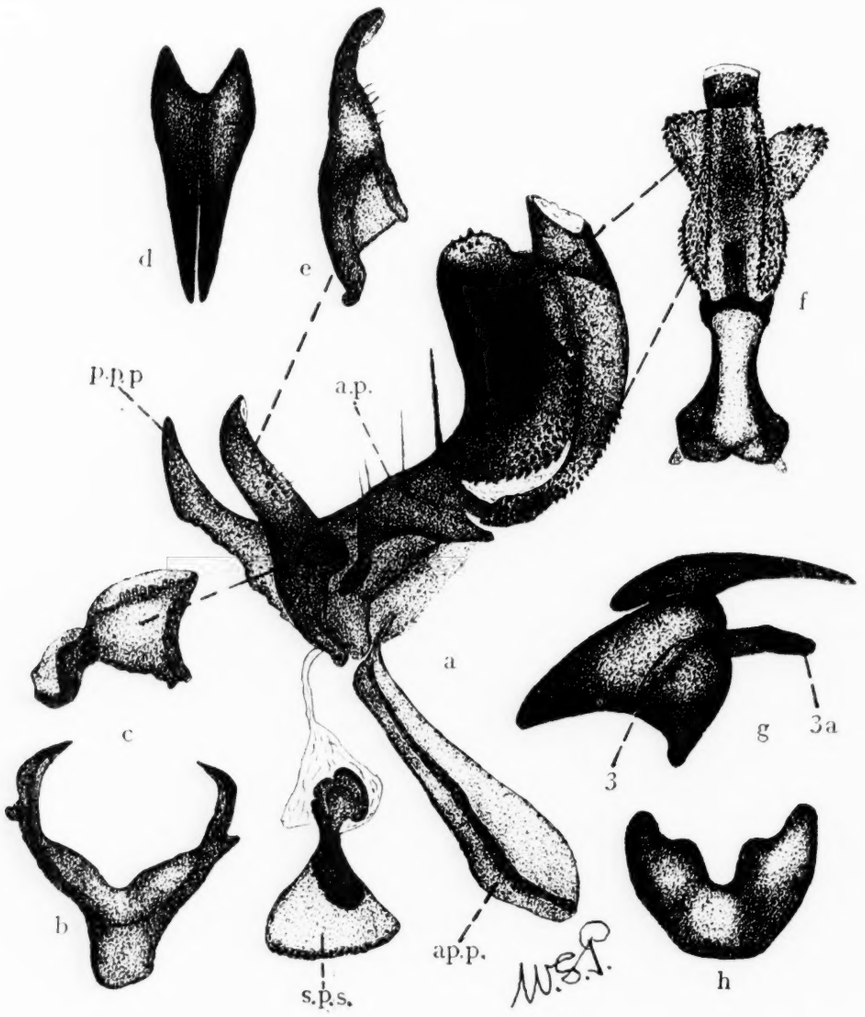


FIG. 3. *a*.—Phallosome, sperm pump sclerite, apodeme, posterior process of phallosome and one paramere of *indica* in side view ; lettering as in fig. 1, *a*. ; *b*.—Ninth tergo-sternum ; *c*.—Anterior part of right paramere ; *d*.—Ventral view of anal cerci ; *e*.—Posterior part of right paramere ; *f*.—Dorsal view of phallosome ; *g*.—Lateral view of tenth tergum showing anal cercus and ninth coxite ; *h*.—Fifth sternum.

Cordylobia (Elephantoloemus) indica Austen. SALIENT DIAGNOSTIC CHARACTERS OF THE TERMINALIA. ♂. As in *anthropophaga*, the ♂ terminalia are small and inconspicuous. Sternum 5 is similar to that of *anthropophaga* ; the lateral lobes are, however, narrower, as shown in fig. 3, *h*. *Ninth Tergo-Sternum*. Fig. 3, *b*. Shorter and wider than that of *anthropophaga*. *Ninth Coxite*. *Distal Segment*. Lateral view. Fig. 3, *g*. The distal segment, like

that of *anthropophaga*, is a lateral clasper, but is longer ; the proximal segment is a stout rod. *Anal Cercus.* Ventral view. Fig. 3, *d.* The anal cercus is shorter and narrower than that of *anthropophaga*, the free pointed end is about half its length ; in lateral view (fig. 3, *g*) the cercus appears more pointed than that of *anthropophaga. Phallosome.* Lateral view. Fig. 3, *a.* Structurally similar to that of *anthropophaga.* Note, for instance, the long sloping ventral surface ending in the longer and more pointed posterior process ; the distal portion is also very similar, and consists of two parts joined proximally by a bar which extends a short distance along the middle line dorsally, as shown in fig. 3, *f* ; the lateral wing-like projections of the ventral part are not so long and prominent as those of *anthropophaga*, but are shorter and broader ; the opening of the ejaculatory duct is wider. *Paramere.* Lateral view. Fig. 3, *a*, *c*, *e*. The two parts of the paramere are strikingly similar to those of the paramere of *anthropophaga.*

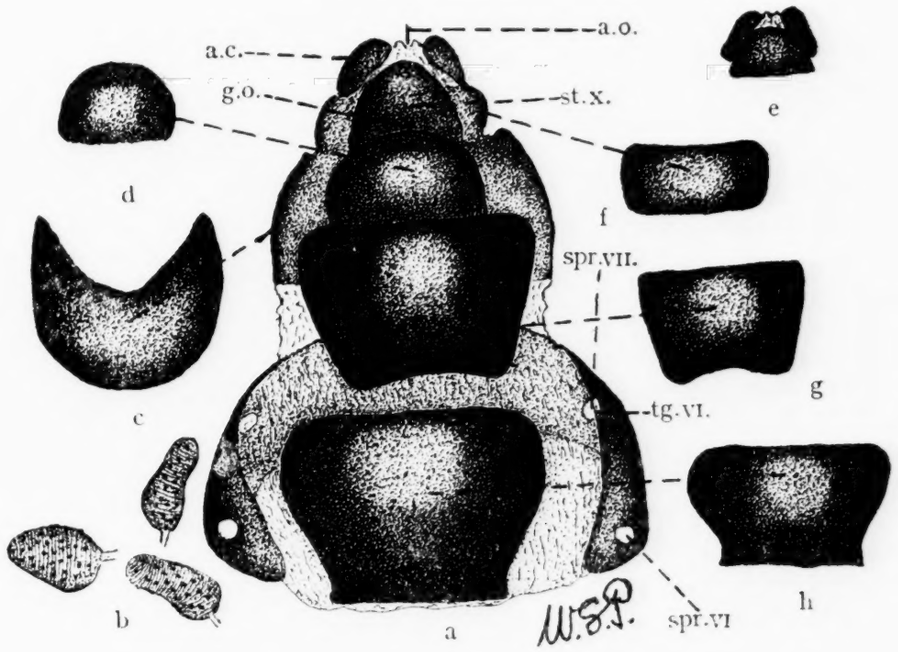


Fig. 4. *a.*—Ventral view of extended ovipositor of *indica* showing genital (*g.o.*) and anal (*a.o.*) openings and sclerites (sternum ten, *st.x.* ; tergum six, *tg.vi.* ; spiracles six and seven, *spr.vi.*, *spr.vii.*) ; *b.*—Spermathecae ; *c.*—Seventh tergum ; *d.*—Ninth sternum ; *e.*—Tenth tergum and anal cerci ; *f.*—Ninth tergum ; *g.*—Seventh sternum ; *h.*—Sixth sternum.

♀. The ♀ terminalia of *indica* are illustrated in Fig. 4, *a.* This ovipositor is shorter and considerably wider than that of *anthropophaga*, which it closely simulates. Note the very large shield-like sternal plates, recalling those of the ovipositor of some of the ♀♀ of the species of *Auchmeromyia.*

Cordylobia (Stasisia) rodhaini Gedoelst. As I have not been able to obtain specimens of the adults of this species, I am unable to illustrate the terminalia. Rodhain and Bequaert (1916), however, have given an excellent illustration of the ♂ terminalia, and a comparison of the parts with those of *anthropophaga* leaves no doubt that this species is a true *Cordylobia*, and is probably closely related to *anthropophaga*. It will be remembered that the larva lives in the skin of small antelopes (*Cephalophus gambianus*, *C. dorsalis*, *C. grimmia*) and of rodents (*Cricetomys gambianus*, etc.) ; it accidentally parasitizes man. Rodhain and Bequaert have given a very full account of this interesting warble fly.

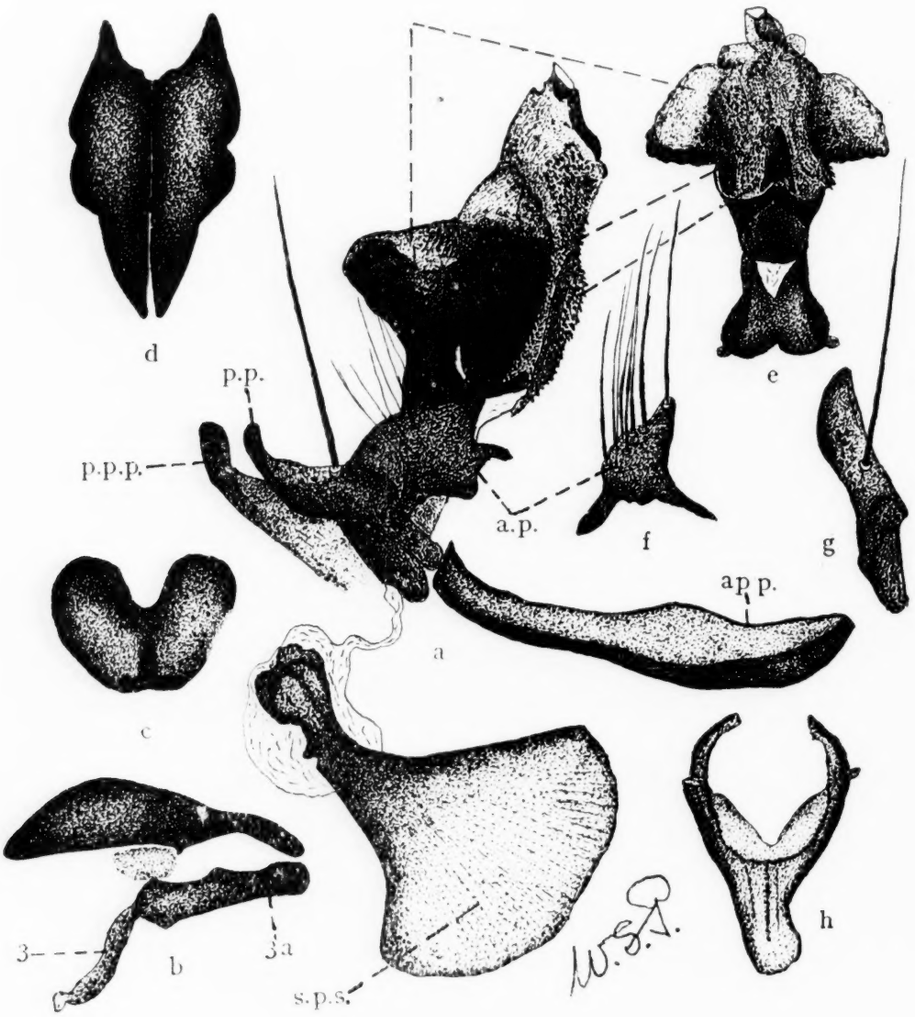


FIG. 5. *a.*—Phallosome, sperm pump sclerite, apodeme, posterior process of phallosome and one paramere of *roubaudi* in side view ; lettering as in fig. 1, *a.* ; *b.*—Anal cercus and ninth coxite in side view ; *c.*—Fifth sternum ; *d.*—Ventral view of anal cerci ; *e.*—Dorsal view of phallosome ; *f.*—Anterior part of right paramere ; *g.*—Posterior part of right paramere. *h.*—Ninth tergo-sternum.

Cordylobia (Neocordylobia) roubaudi Villeneuve. SALIENT DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. As in the two previous species the ♂ terminalia are small and inconspicuous. Sternum 5 (fig. 5, *c*) is very similar to that of *anthropophaga*. *Ninth Tergo-Sternum*. As in fig. 5, *h*. *Ninth Coxite. Distal Segment*. Lateral view. Fig. 5, *b*. Longer and wider than the distal segment of *anthropophaga* and *indica*, the distal end bent inwards, forming a lateral clasper ; proximal segment a stout rod, as in fig. 5, *b*. *Anal Cercus*. Ventral view. Fig. 5, *d*. Short, broad and ending in a blunt point, the anterior half free, the remainder joined to its fellow ; lateral view as in fig. 5, *b*. *Phallosome*. Lateral view. Fig. 5, *a*. Short and broad, the sloping ventral surface of proximal end shorter than in the phallosome of *anthropophaga* and *indica* (more like that of *Auchmeromyia*). As in the two previous species, the distal portion consists of two parts joined proximally by a chitinous bar which extends a short distance in the middle line on the dorsal side, forming an incomplete shaft (fig. 5, *e*). The ventral portion of the distal end is wider than that of either *anthropophaga* or *indica*, and projects laterally on each side as a large ear-like chitinized flap (fig. 5, *e*); there is also a densely chitinized spined plate on each side (fig. 5, *a*, *e*). The dorsal portion consists of two spined flaps, one on each side of the middle line ; the longer ejaculatory duct projects well beyond the end of the phallosome, and is supported distally by a median plate and chitinous collar (fig. 5, *a*, *e*). *Paramere*. Lateral view. Fig. 5, *a*, *f*, *g*. The two parts of the paramere are very like those of *anthropophaga* and *indica*.

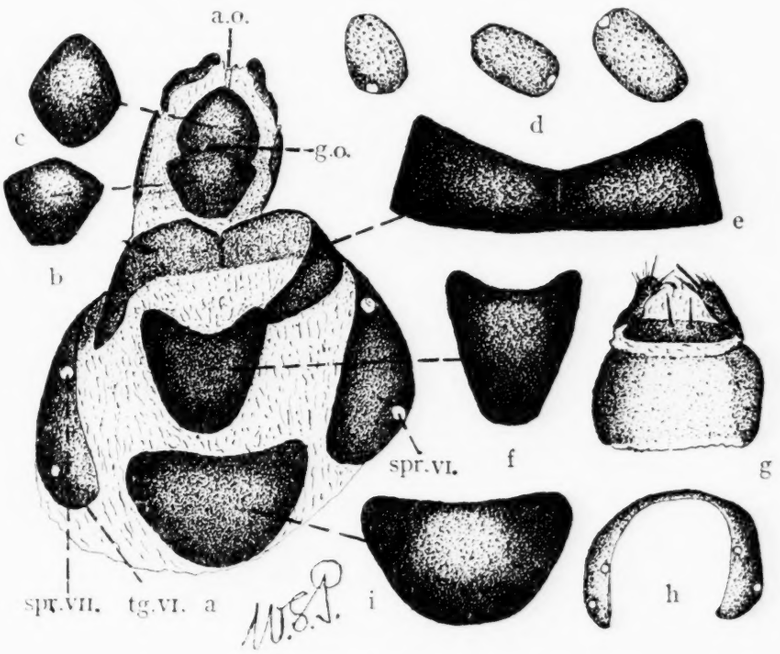


FIG. 6. *a*.—Ventral view of extended ovipositor of *roubaudi* ; lettering as in fig. 4, *a*. ; *b*.—Ninth sternum ; *c*.—Tenth sternum ; *d*.—Spermathecae ; *e*.—Seventh tergum ; *f*.—Seventh sternum ; *g*.—Tenth tergum and anal cerci ; *h*.—Sixth tergum ; *i*.—Sixth sternum.

♀. The ♀ terminalia of *roubaudi* are illustrated in fig. 6. Except for some minor differences in size and shape, the sclerites of the ovipositor are similar to those of *anthropophaga* and *indica*.

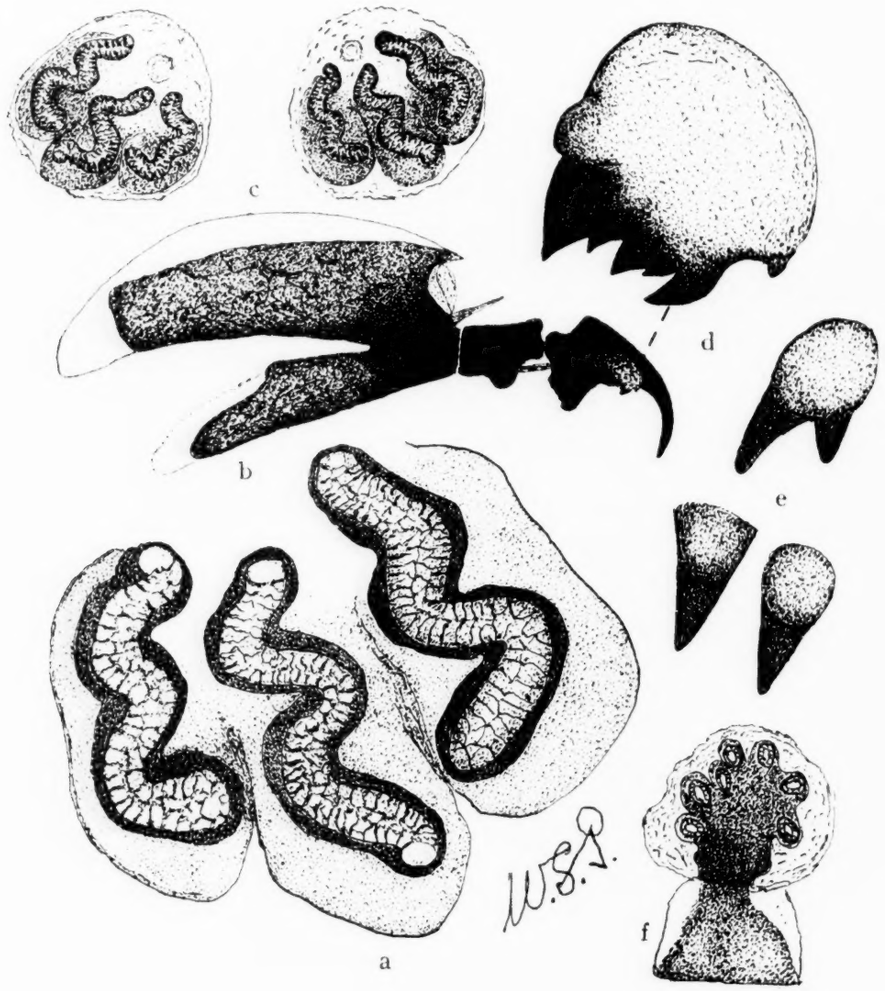


FIG. 7. *a*.—One spiracular plate of the third larva of *anthropophaga* enlarged ; *b*.—Cephalopharyngeal skeleton of third larva of same ; *c*.—Spiracular plates of third larva of same ; *d*.—Lateral cutting plate of third larva of same ; *e*.—Three dermal spines of third larva of same ; *f*.—Anterior spiracle of third larva of same.

SALIENT DIAGNOSTIC CHARACTERS OF THE LARVA OF CORDYLOBIA. Figs. 7; 8. The larva of *anthropophaga*, *indica*, *intonsa* and *rodhaini* have been fully described by different authors, and I have been able to study all four ; it will be remembered that the larva of *roubaudi* is as yet unknown. The general character common to the four larvae noted above is that they are rather squat, the distal end rounded, and the thoracic and most of the abdominal segments armed with stout backwardly directed spines arranged in an irregular manner ; in the third stage larva of *anthropophaga* and *intonsa* the spines are more

numerous (and are present on all the segments) than in the larva of *rodhaini* and *indica*. This arrangement of these dermal spines is in marked contrast to the regular segmentally arranged belts of spines of the third stage larvae of the Calliphorinae, which are very well developed in the parasitic forms such as *Chrysomyia bezziana* and *Cochliomyia americana* and others. The cephalopharyngeal skeleton of the larva is similar in the species examined, differing only in minor details ; the several plates are massive, and in the third stage the oral hooks are stout, rather bluntly pointed (except in the larva of *anthropophaga*), somewhat flattened dorso-ventrally, the lateral margins sharp. As far as is known, the first stage larva has a cutting plate which is armed with strong teeth on each side at the end of the cephalopharyngeal skeleton, and is used by it to cut the skin and allow of rapid entrance ; the method of penetrating the skin has been fully described in the case of the first stage larva of *anthropophaga* by Blacklock and Thompson (1923). In the third stage larva there is a flat toothed plate directed dorso-ventrally on the outer side of each oral hook, as illustrated in figs. 7, *b* ; 8, *a*, *e*. A comparison of the cutting or tearing plate in the larva of *A. luteola* and *A. chaerophaga* (see my earlier paper, 1935) with that of the larva of *anthropophaga*, *indica* and *intonsa* clearly shows that they are very similar ; in these larvae of *Cordylobia*, which are already buried in the skin, these plates must, I believe, be used to tear the tissues. The anterior spiracles of the third stage larva are alike, consisting of a rather wide membraneous stalk with short finger-like processes arranged somewhat irregularly and bearing an opening at the end (figs. 7, *f* ; 8, *g*, *k*). The posterior spiracles, too, are very similar in these larvae (figs. 7, *a*, *c* ; 8, *b*, *d*, *h*, *j*). The slits are either short and straight or longer and curled (*anthropophaga*, *rodhaini*). The peritreme is not well developed.

The Species of Cordylobia, Their Relationships to Each Other and the Systematic Position of the Genus. The comparative study of the terminalia of the three species (*anthropophaga*, *indica* and *roubaudi*) at present available can leave no doubt that they are related and belong to the genus *Cordylobia*. The study of the third stage larva of two of these (*anthropophaga* and *indica*) amply confirms this conclusion. A study of the illustrations of the parts, all of which are drawn to scale, will, I think, convince the reader of the accuracy of this conclusion. Although it has not been possible to study the terminalia of *rodhaini*, the excellent drawing of the terminalia of the ♂ by Rodhain and Bequaert, and my own study of the larva (sufficient material to illustrate its characters is not available), confirm the conclusion that this species too is a typical *Cordylobia*, and that it is closely allied to *anthropophaga* ; there is, therefore, no need for the erection of the genus *Stasisia* Surcouf for its reception. There remains the species *intonsa*, whose larva, it will be remembered, lives in the skin of the foot of the carabao in the Philippine Islands. I have unfortunately been unable to obtain specimens of the adults. Professor L. B. Uichanco, Department of Entomology, Agricultural College, Laguna,

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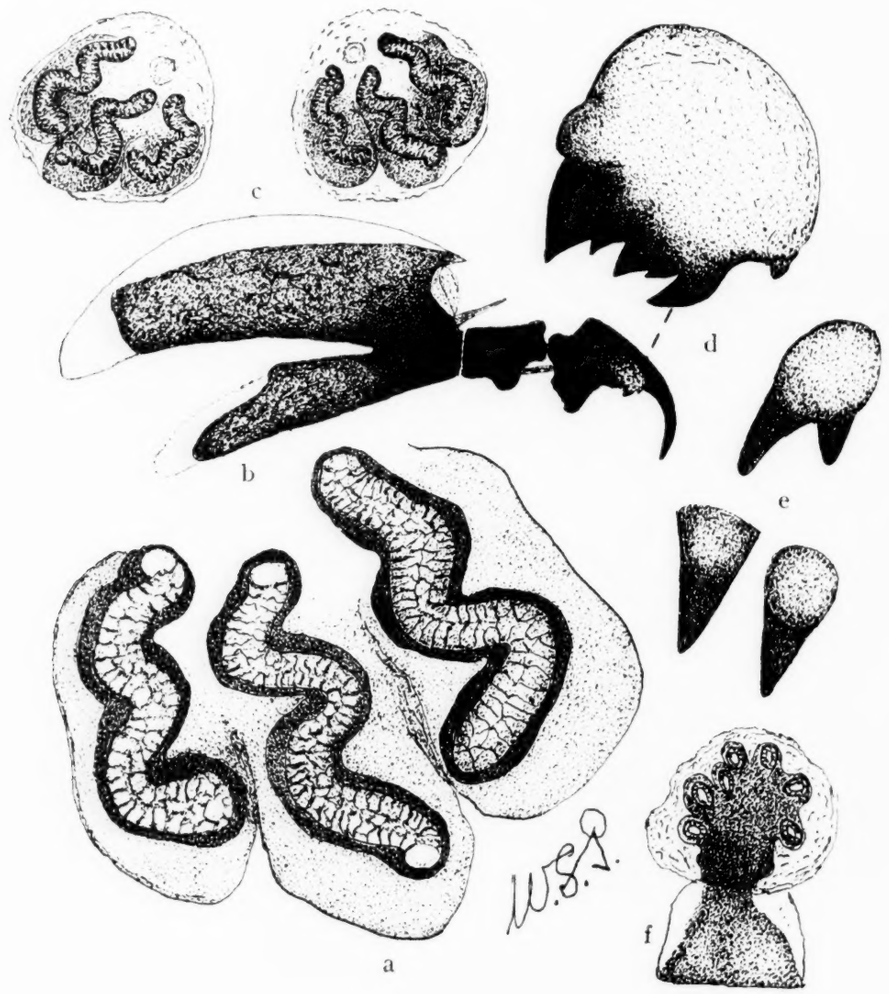


FIG. 7. *a*.—One spiracular plate of the third larva of *anthropophaga* enlarged ; *b*.—Cephalopharyngeal skeleton of third larva of same ; *c*.—Spiracular plates of third larva of same ; *d*.—Lateral cutting plate of third larva of same ; *e*.—Three dermal spines of third larva of same ; *f*.—Anterior spiracle of third larva of same.

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Philippine Islands, has been on the look-out for this interesting fly since 1925, and he tells me that up to the present he has not been able to locate any cases of foot-myiasis in cows and carabaos caused by its larva. Since 1922–23, when Woodworth and Ashcraft studied it, this fly apears to have disappeared from the locality. Professor Uichanco very kindly gave me some specimens of third

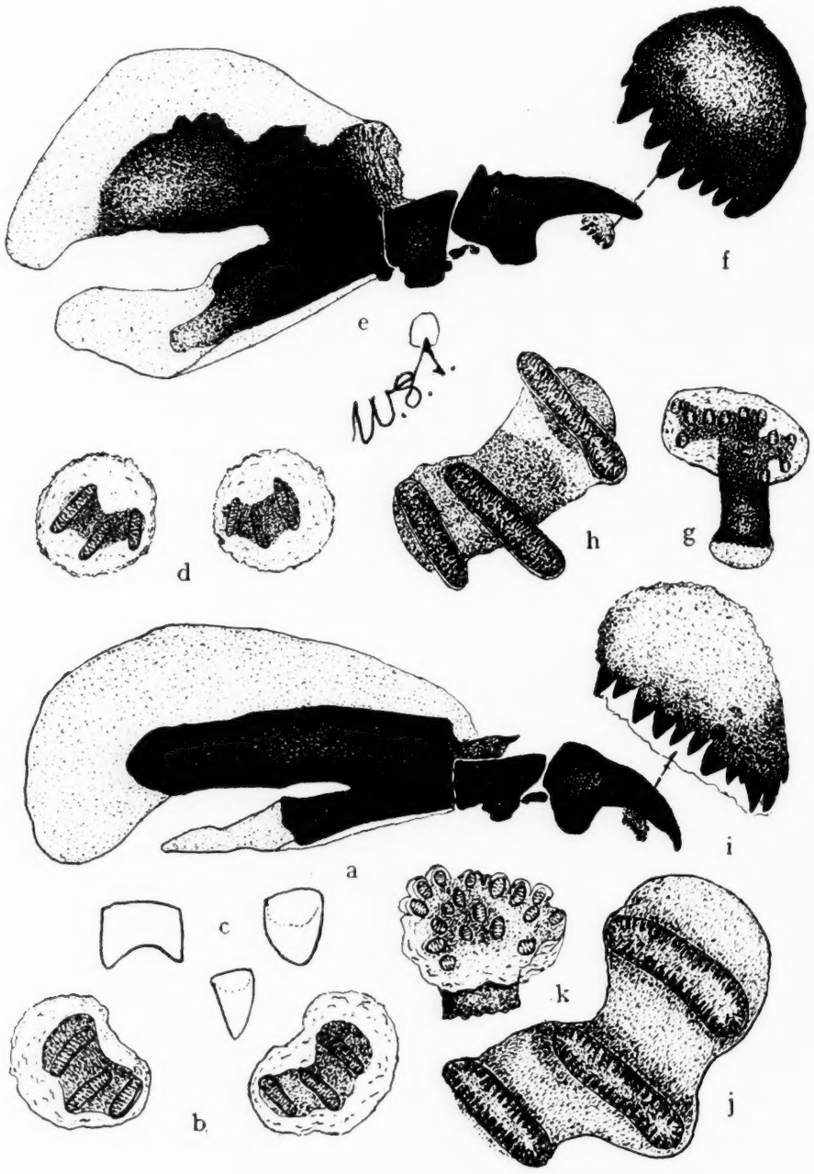


FIG. 8. *a.*—Cephalopharyngeal skeleton of third larva of *intonsa*; cutting plate shown enlarged; *b.*—Spiracular plates of third larva of same; *c.*—Dermal spines of third larva of same; *d.*—Spiracular plates of third larva of *indica*; *e.*—Cephalopharyngeal skeleton of third larva of same; cutting plate shown enlarged; *f.*—Lateral cutting plate of third larva of same enlarged; *g.*—Anterior spiracle of third larva of same; *h.*—One spiracular plate of third larva of same; *i.*—Lateral cutting plate of third larva of *intonsa* enlarged; *j.*—One spiracular plate of third larva of same enlarged; *k.*—Anterior spiracle of third larva of same enlarged.

stage larvae, and the study of the characters of this stage (fig. 8) leads me to the provisional conclusion that this fly is also a true *Cordylobia*.

The species *roubaudi* Villeneuve is of special interest. It is commonly found in and around the burrows of the Aard Vark, in company with *Auchmeromyia praegrandis* and *A. bequaerti*. A comparative study of the terminalia, and particularly of the ♂ terminalia, with those of *anthropophaga* and *indica*, on the one hand, and with those of *Auchmeromyia* (especially *A. boueti* and *A. praegrandis*) on the other, leads me to the conclusion that, although this species is a true *Cordylobia*, it links together the two genera, thus establishing with certainty the exact systematic position of the genus *Cordylobia*. In the first place, it will be noted that the phallosome in *Cordylobia anthropophaga* has characters in common with that of the Auchmeromyias ; the distal portion is divided into two parts connected basally with a chitinous bar and distally by membrane ; in *Auchmeromyia* the bar is continued in the middle line along the dorsal part, to form a median shaft carrying the ejaculatory duct ; in *Cordylobia* this bar, though present, is incomplete. The paramere in the two is also very similar ; and the ♀ terminalia, too, are alike. Tergum 5 in the ♀ *Auchmeromyia* is modified for a special method of oviposition, the abdomen being dragged along to make a furrow in which the egg or eggs are laid.

The method of oviposition of *C. anthropophaga*, on the other hand, is quite different, and has been described by Blacklock and Thompson (1923) as follows : 'The fly, having alighted on the surface of the sand, and having found a suitable area, digs with the tip of the abdomen a small cavity in the sand, backing slightly and curving the abdomen downwards to enable it to do so ; the ovipositor is then extruded and pushed into the sand at the bottom of the cavity.' Tergum 5 is, therefore, not modified in *anthropophaga* to form a plough as it is in *Auchmeromyia*. *Cordylobia indica* lays its eggs directly on the surface of the skin of the elephant ; it is not known where the ♀ *roubaudi* lays its eggs.

A comparison of the phallosome of *roubaudi* with that of *A. boueti* and *A. praegrandis* (see my earlier paper, 1935) will, I think, show that the former has some characters in common with that of each of the latter. The longer dorsal portion in *roubaudi* carrying the ejaculatory duct, the partially formed median shaft, the ventral flaps, the very large sperm pump sclerite, and the structure of the paramere are some of the characters which suggest relationships. The ♂ *roubaudi* is dichoptic, like the ♂ of *rodhaini*. I believe, then, that *Cordylobia* is closely related to *Auchmeromyia*, the two being linked together by the species *C. roubaudi*, which, though a true *Cordylobia*, suggests relationships with *Auchmeromyia*, especially with *boueti* and *praegrandis*, two species which are more closely associated with the Aard Vark than the wart-hog. I believe also that both genera have had a common origin. This study further suggests that the larva of *roubaudi* is in all probability a dermal parasite of the Aard Vark.

It is, I think, worth pointing out here that these important conclusions

regarding the species of *Cordylobia*, their relationships to each other and the systematic position of the genus could not possibly have been elucidated by the study of the external characters used by Surcouf in his monograph. The use of such characters as the arrangement of hairs and bristles, shape of the head, height of the peristome, the structure of the arista, etc., would inevitably leave a large margin of doubt whether a given species was a true *Cordylobia* or not ; and as a result we find that the four species *rodhaini*, *intonsa*, *indica* and *roubaudi* have been placed in the genera *Stasisia*, *Booponus*, *Elephantoloemus* and *Neocordylobia* respectively. The final, and, in my opinion, the certain, decision as to the true systematic position and relationships of *indica* and *roubaudi* could only have been reached as a result of the study of the terminalia.

GENUS CORDYLOBIA GRÜNBERG (EMENDED PATTON 1936)

GENERIC CHARACTERS. LARVA. Cutaneous parasite of mammals, the first stage larva being equipped for entering the unbroken skin. THIRD STAGE LARVA. Cuticle armed with spines arranged irregularly on the thoracic and abdominal segments. Oral hooks of equal length, each with a lateral toothed plate. Anterior spiracles consisting of membraneous stalks bearing a number of short finger-like processes, each with an opening. Eighth segment rounded. Posterior spiracles small, not widely separated, peritreme mainly membraneous, the three breathing slits either short and straight, or longer and sinuous, and directed downwards and outwards. ADULTS. Male either holoptic or dichoptic. Arista either with long hairs on both surfaces, or with short hairs on the dorsal side only, the sixth segment then stout as in *indica* and *intonsa*. Chaetotaxy much as in *Auchmeromyia*; palps and proboscis as in *Auchmeromyia*. Squama bare. WING. *Venation*. As in *Auchmeromyia*. M_{1+2} with a rounded bend forwards in its distal third, then running almost parallel with R_{4+5} and ending close to it, so that cell R_5 has a bottle-neck opening. MALE TERMINALIA. ANAL CERCI. Free distally (about half), remainder joined to fellow, end pointed. NINTH COXITE. Distal segment forming a lateral clasper, turned inwards and with blunt end. PHALLOSOME. Short, proximal ventral surface sloping back to posterior process ; distal portion consisting of two parts, as in *Auchmeromyia*, joined proximally by a chitinous bar, the median shaft incomplete, and its continuation either short or fairly long, bearing ejaculatory duct at end. FEMALE TERMINALIA. Tergum 5 not modified to form a plough, as in ♀ *Auchmeromyia*. OVIPOSITOR. Relatively short, with complete tergal and sternal plates ; tergum six consists of a single plate. *Chitinous Plate of Uterus*. Wanting. SPERMATHECAE. Short, wide and somewhat pear-shaped.

SUMMARY

1. The ♂ and ♀ terminalia of *anthropophaga*, *indica* and *roubaudi* are illustrated, and their main diagnostic characters are noted. The comparative study of the terminalia of these three species leaves no doubt that *indica* and

roubaudi are related to *anthropophaga*, and belong to the genus *Cordylobia*. A comparative study of the ♂ terminalia of *roubaudi* with those of *Auchmeromyia*, especially of *A. boueti* and *A. praegrandis* on the one hand, and of *Cordylobia anthropophaga* on the other, points to this species being a link between the two genera. Rodhain and Bequaert's illustration of the ♂ terminalia of *rodhaini* shows it to be a true *Cordylobia*. The study of the larva of *intonsa* Aldrich suggests that it, too, is a *Cordylobia*.

2. The larva of each of the four species *anthropophaga*, *indica*, *intonsa* and *rodhaini* is a dermal parasite, and all four possess many structural characters in common.

3. The genus is re-defined, and as it is closely allied to *Auchmeromyia* it is here placed with it in the subfamily Auchmeromyinae.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS GLOSSINA WIEDEMANN BASED ON A COMPARATIVE STUDY OF THE MALE AND FEMALE TERMINALIA

(*Continued from Vol.* 28, *page* 322)

BY

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INTRODUCTION

In an earlier paper (1934) I illustrated and described the segmentation of the abdomen in *Glossina* and the ♂ and ♀ terminalia of *longipennis* Corti. As there is a demand for further parts of this revision of the species, I propose to complete them as soon as possible. In the present paper I shall describe the ♂ and ♀ terminalia of several species of the *fusca* group, giving at the same time the salient diagnostic characters for their identification, together with short notes on their distribution and importance. I shall also illustrate the antenna of the ♂ of each species (all drawn to the same scale), and depict the characters of the peg of the second segment and of the sensoria in the third segment. In *Glossina* there are two characteristic sensoria towards the base of the third segment, one of which is apparently the more closely associated with the arista, and is a compound organ with a duct-like channel opening close to the base of the arista on its inner side. The other sensorium is circular and opens on the outer surface ; in each case these sensoria are shown enlarged. It is necessary also to refer to one other structure and to make a correction. The sclerite which attaches, or binds, the phallosome to the parameres, and both to the ninth tergo-sternum, and which is present in almost all the higher Diptera, often provides an additional diagnostic character in *Glossina* ; in *tabaniformis*, for instance, it is shaped like a boot, while in *brevipalpis* it is very prominent. This sclerite is referred to here as the median binding sclerite. Lastly, it is necessary to correct what I believe is an error in the interpretation of sternum 9 in ♀ *Glossina*. The plate attached anteriorly to sternum 10 was labelled sternum 9 in the case of the ♀ terminalia of *longipennis*. A comparative study of the ♀ terminalia of *Glossina* with those of the ♀ *Sarcophaga* has led to the conclusion that this plate is most probably a part of sternum 10 and not sternum 9. As in *Sarcophaga*, sternum 9 appears to have become fused with sternum 7, and in *Glossina* it is entirely membraneous. The more recent literature relating to the species of *Glossina* will be given at the end of the last part of this series.

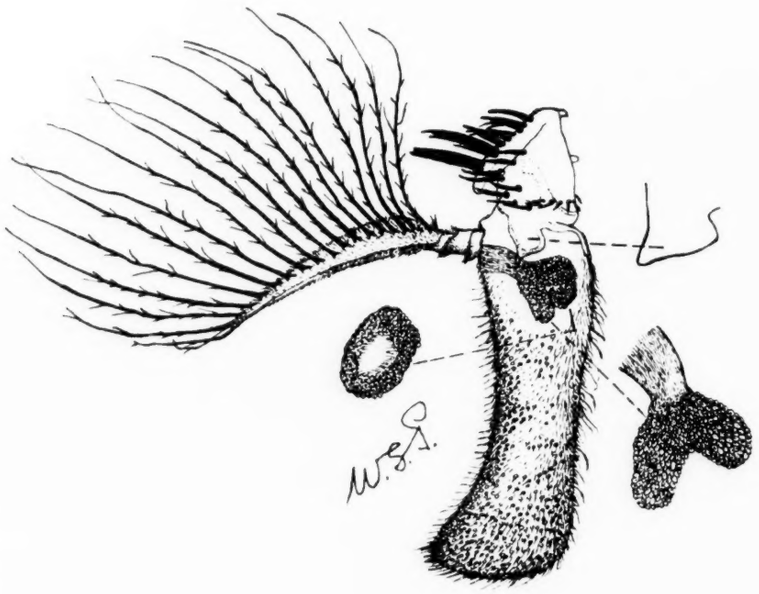


FIG. 1. Antenna of ♂ *longipennis*, the peg of the second segment and the sensoria of the third shown enlarged at side ; note in this species that the aristal hairs have short branches and that almost half the distal end of each hair is bare.

SPECIES OF THE FUSCA GROUP

Glossina longipennis Corti. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A large species, measuring from 11 to 14 mm. in length, with pale wings. Ocellar triangle dark, forming a conspicuous spot. Antenna (fig. 1) relatively small, aristal hairs with very short branches and about half their distal ends bare. Mesonotum with four dark-brown spots, a pair anterior and a pair posterior to the mesonotal suture ; a pair of incomplete dark admedian stripes sometimes extending behind suture. Abdomen buff, especially terga 1 and 2, the remainder darker. DIAGNOSTIC CHARACTERS OF TERMINALIA. The illustrations of the teminalia are in my first paper (1934). ♂. *Anal Cerci.* Long rod-like plates completely separated and each ending in a long, wide, bluntly pointed, dark, claw-like process. *Phallosome.* Proximal part with a characteristic spined flap and a long blunt process projecting upwards ; distal end with a pair of bent prong-like processes ; median binding sclerite not raised. *Paramere.* Not conspicuous and gently sloping to end. ♀. Tergum 9 consists of a pair of somewhat triangular plates lightly joined and almost completely filling the space between postero-lateral margins of tergum 7 ; their antero-internal halves are more strongly chitinized and bear the bristles. The signum is a strong folded chitinous plate.

DISTRIBUTION. HABITS. IMPORTANCE. This species was discovered in 1893 by Bottego on the banks of the Welmal, a tributary of the Juba River in Abyssinia. It is now known to be limited to an area in N.E. Africa from about 4° N. to 4° S., and from 33° E. to 47° E. As noted above, it extends northwards

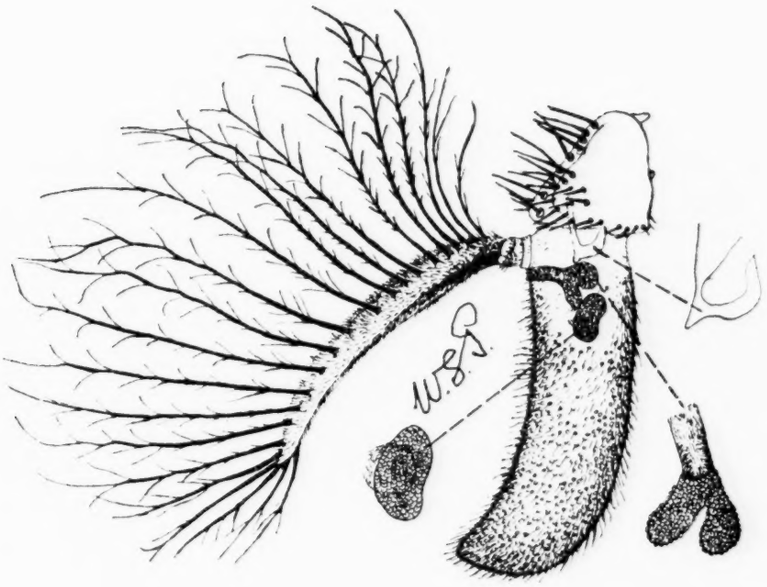


FIG. 2. Antenna of ♂ *fusca*, the peg and sensoria shown enlarged.

into Abyssinia and Italian Somaliland, but its northern limit here is not definitely known. It has been recorded by King (1912) from two localities in the Mongalla Province, east of the Nile in the Sudan. Anderson (1921) found it to be common in the moist swampy flats and on the bank of the River Uaso Nyiro in the Masai

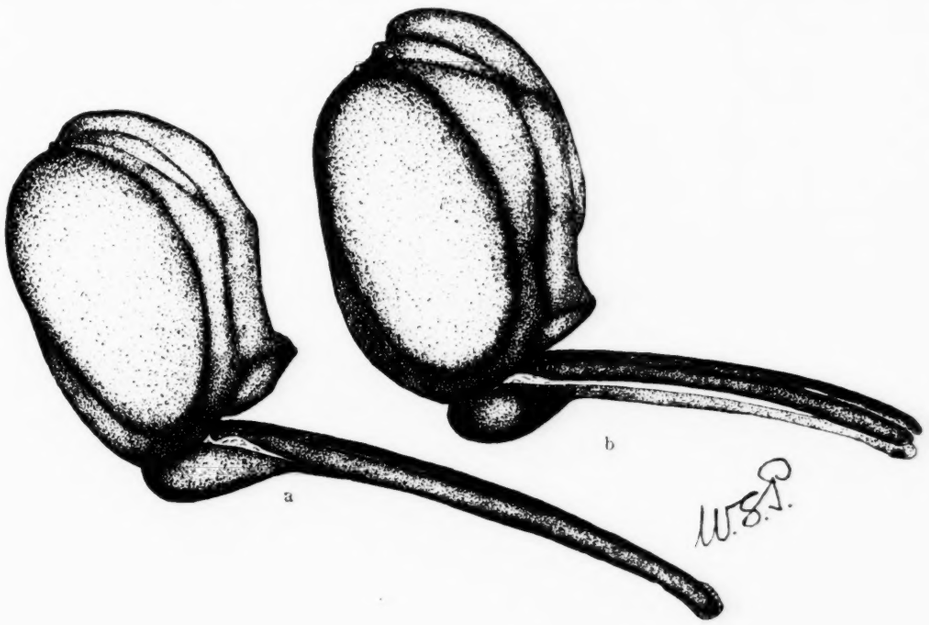


FIG. 3. *a.*—Head of ♂ *fusca* in side view to show the long palps (much longer than width of head); this is an example of a species with long palps; *b.*—Head of ♂ *brevipalpis* to show the much shorter palps (not longer than the width of the head); this is an example of a species with short palps.

Reserve. It has often been caught in railway carriages. It is a desert or semi-desert species ; in higher regions it is usually found between rivers, but rarely on their banks. It is mainly a nocturnal feeder, especially the ♂, which is the commoner sex to be captured. Although it has been proved to be a transmitter of *T. dimorphon* (Ross, 1913) experimentally, it is doubtful if it plays any large part in spreading this and allied trypanosomes.

Glossina fusca Walker. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A large species, with the characteristic

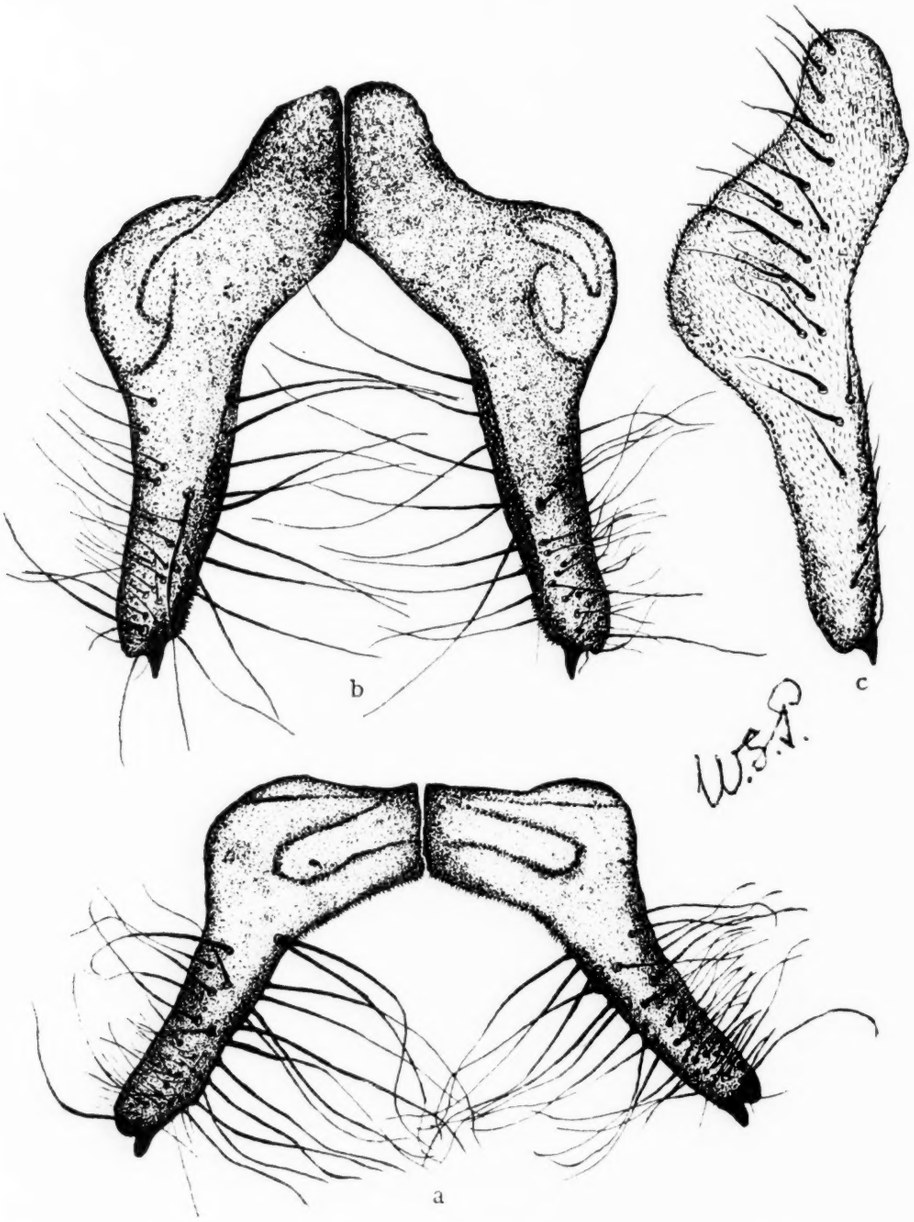


FIG. 4. *a.*—Ventral view of extended anal cerci of *fusca* var. *congolensis* for comparison with those of *fusca*, both drawn to the same scale ; *b.*—Ventral view of extended anal cerci of *fusca* ; *c.*—Dorsal view of one anal cercus of same.

Glossina thoracic markings consisting of dark brown stripes and spots, and with dusky wings ; it measures from 8·6 mm. to 13·4 mm. Aristal hairs (fig. 2) with relatively short branches extending almost to end. Palps (fig. 3, *a*) long and relatively slender (about one-third longer than width of head). Thoracic markings distinct ; wings fairly dusky. Abdomen without distinct banding ; terga 1 and 2 lighter than remainder.

Diagnostic Characters of Terminalia. ♂. *Anal Cerci.* Fig. 4, *b*, *c*. Long, rounded, rod-like plates, each with a short, black, pointed process at end. *Phallosome.* Figs. 5 ; 6. With a characteristic, broad, finely serrated (saw-like) plate (fig. 6, *d*) with a short process at its base ; at more distal end of phallosome there is a calliper-like sclerite with a long and a shorter pointed bent process. Median binding sclerite (fig. 6, *a*) raised and rounded in side view. ♀. Fig. 6, *e*. Two plates of tergum 9 subcrescentic with bristles distributed over almost entire surface. Signum as in fig. 6, *c*.

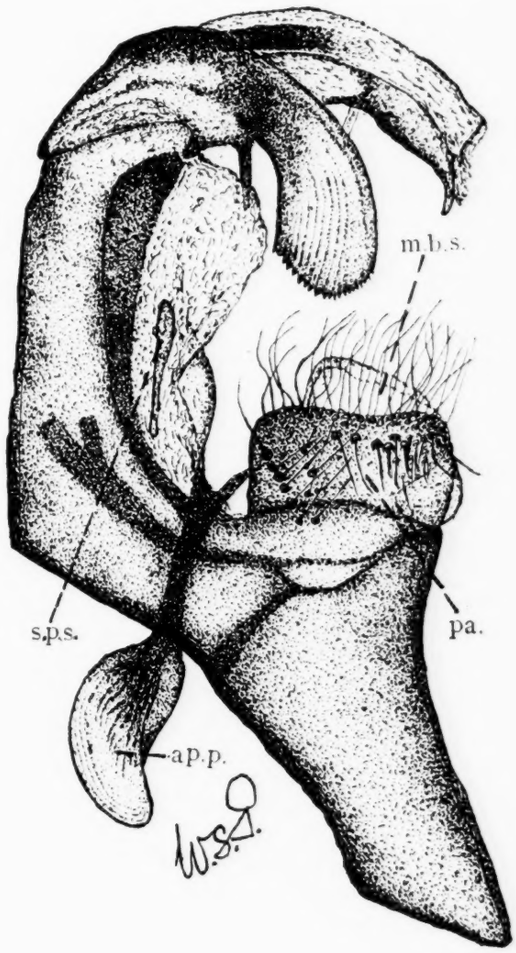


Fig. 5. Lateral view of ninth tergo-sternum of *fusca* showing phallosome embedded in its dorsal concavity ; *pa.*, paramere ; *ap.p.*, apodeme of phallosome ; *m.b.s.*, median binding sclerite ; *s.p.s.*, sperm pump sclerite.

DISTRIBUTION. HABITS. IMPORTANCE. This is a Western and Central African species, extending across the continent to the east, where it is replaced by the variety *congolensis* Newstead and Evans. *G. fusca* is found in French Guinea, is common and widely distributed in Sierra Leone, is found in Northern Liberia, Ivory Coast, Gold Coast, Dahomey, Nigeria, Cameroons, down to Angola on the west and across the Belgian Congo to Uganda. Its exact eastern limits are not definitely known ; some at least of the records from Uganda, the Eastern Provinces of the Belgian Congo, from Kenya and Tanganyika almost certainly refer to the variety *congolensis*. *G. fusca* is essentially a forest species, and is common in dense vegetation and forest along the courses of rivers and streams ; it has also been recorded from forest country far from water,

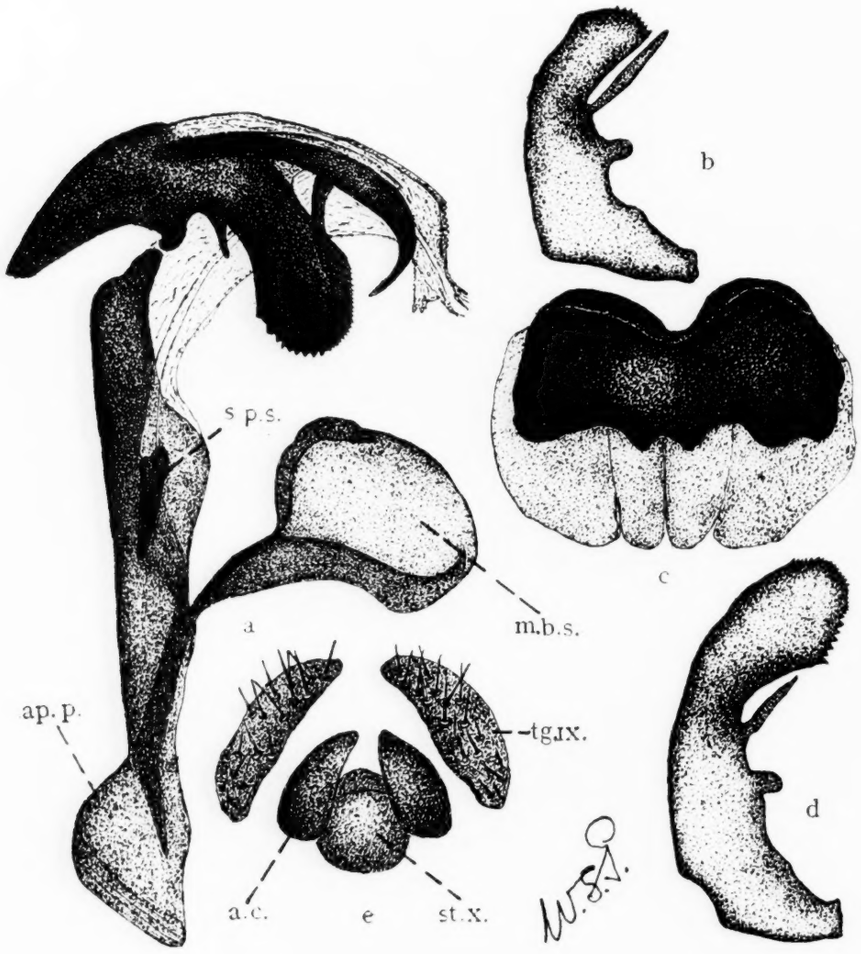


FIG. 6. *a.*—Lateral view of phallosome of *fusca* dissected out of ninth tergo-sternum and showing the apodeme (*ap.p.*), median binding sclerite (*m.b.s.*) and sperm pump sclerite (*s.p.s.*) ; note the broad serrated plate with smaller process at its base ; *b.*—The same serrated plate of phallosome of *fusca* var. *congolensis* ; note that it is narrower and the accessory basal process is longer in this variety ; *c.*—Signum of *fusca* ; *d.*—Serrated plate of phallosome of *fusca* for comparison with that of *fusca* var. *congolensis* ; this structure affords an important diagnostic character for distinguishing *fusca* from its variety *congolensis* ; *e.*—Terminalia of ♀ *fusca* ; *a.c.*, anal cerci ; *st.x.*, tenth sternum ; *tg.ix.*, ninth tergum.

and in these cases may have been carried there by animals. In Kenya it is said to occur in dry, rather sparsely wooded country (Bevan, 1925). It is generally considered to be more closely associated with elephants, buffaloes and cattle than with other animals. It is crepuscular in habit and is seldom caught at midday ; it has been recorded biting late at night. It has been proved to be capable of transmitting *T. gambiense* (Ross, 1908). Schwetz (1928) considers that *fusca* (and var. *congolensis*) is the vector of animal trypanosomes (*T. vivax* and *T. congolense*) in the Belgian Congo.

Glossina fusca var. **congolensis** Newstead and Evans. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. Very similar to *fusca* Walker, but wings if anything darker. It is necessary to examine the ♂ terminalia to be sure of the identification. DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Fig. 3, *a.* Shorter than those of *fusca* and process at end perhaps blunter ; otherwise practically indistinguishable from those of *fusca. Phallosome.* Fig. 6, *b.* Also very similar to that of *fusca*, but in most cases readily distinguished by the shorter, narrower, serrated process and the longer proximal rod-like process reaching to the end of the serrated blade ; the distal part of the calliper-like process is half the length of the proximal. It should be remembered that intermediate forms have been recorded by Newstead, in which these differences are not marked.

DISTRIBUTION. HABITS. IMPORTANCE. This variety was described from specimens captured by Dr. Schwetz in the Katompo and the Lomami-Kisengwa Districts of the Belgian Congo. Dr. Schwetz, the well-known authority on

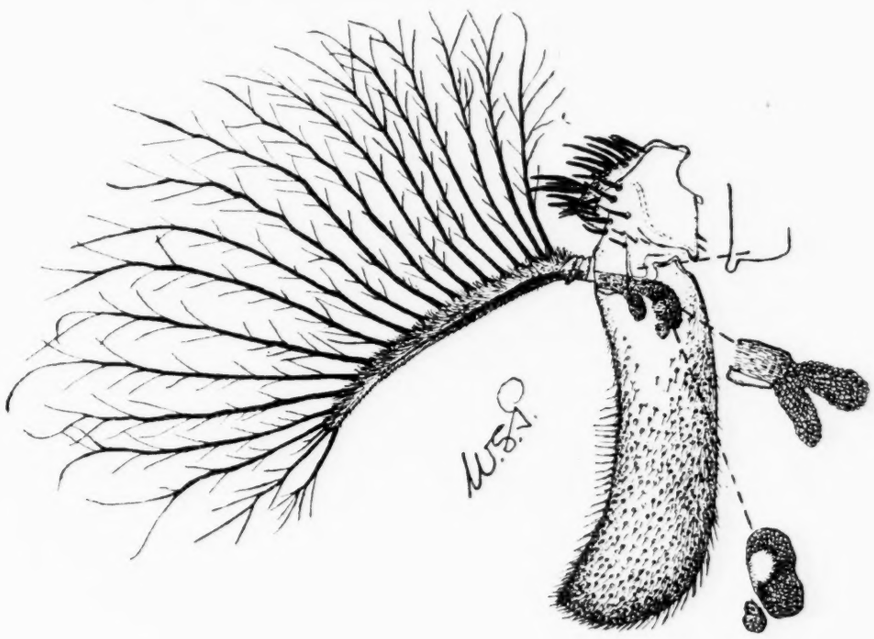


FIG. 7. Antenna of ♂ *fuscipleuris* with peg and sensoria shown enlarged.

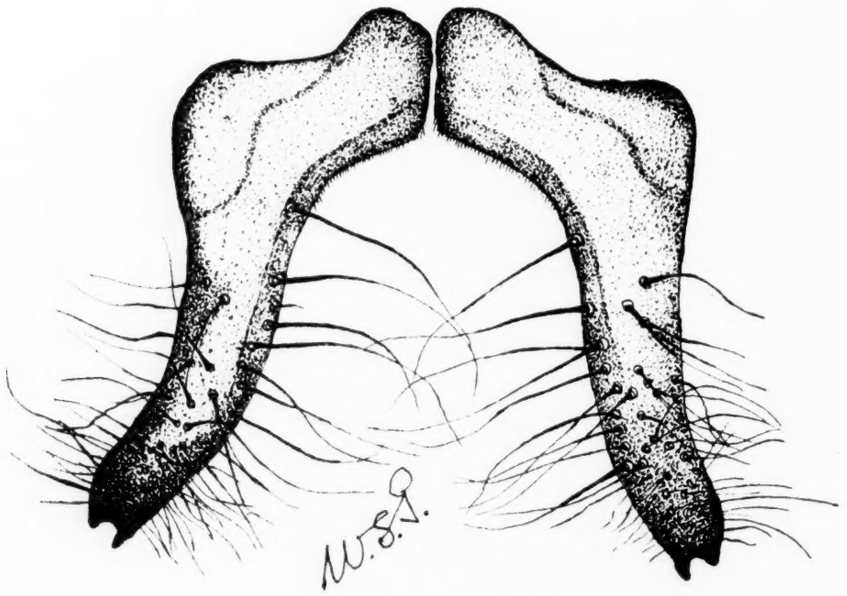


FIG. 8. Ventral view of extended anal cerci of *fuscipleuris*.

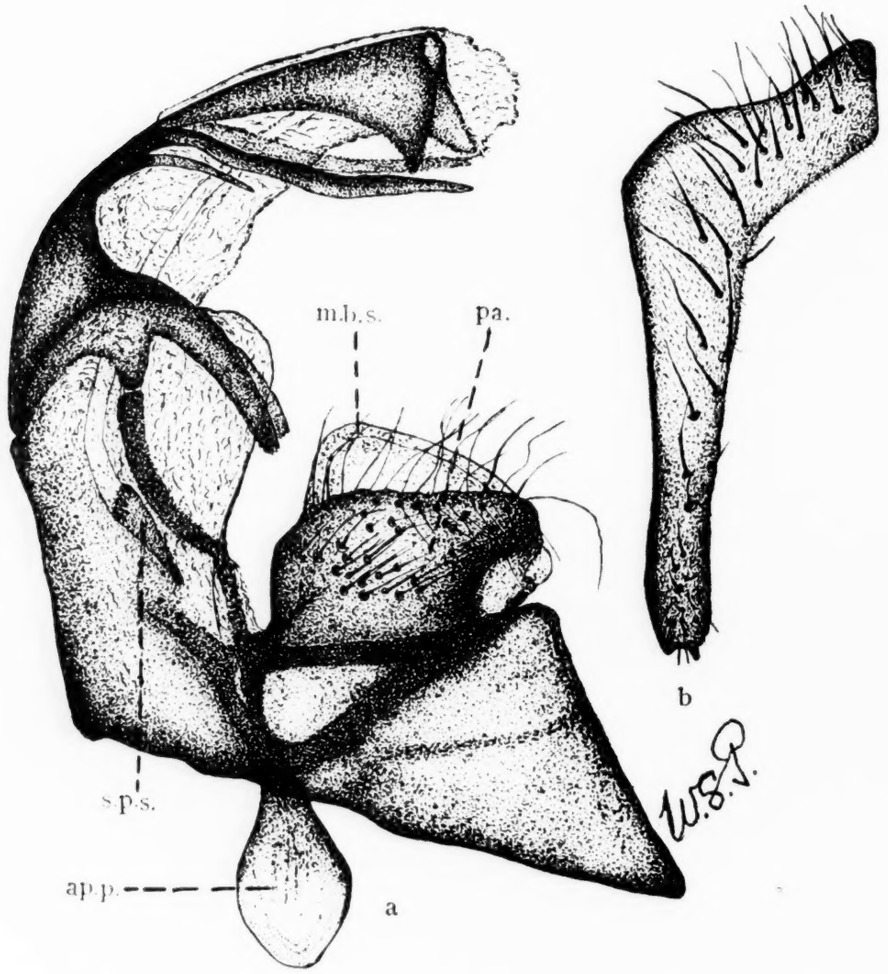


FIG. 9. *a*.—Lateral view of ninth tergo-sternum of *fuscipleuris* showing phallosome embedded in its dorsal concavity ; lettering as in fig. 6, *a* ; *b*.—Dorsal view of anal cercus of same.

the species of *Glossina* found in the Belgian Congo, who has studied their distribution, habits, etc., says that *fusca* (and var. *congolensis*) is the most widely distributed species in the Belgian Congo.

Glossina fuscipleuris Austen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A large dusky species closely resembling, and commonly confused with, *fusca* Walker (and var. *congolensis*). Antenna (fig. 7) with long aristal hairs, and long branches reaching to ends ; palps long. Thorax with pleurae dark-grey and distinctly darker than those of *fusca* and var. *congolensis* ; alar tubercle (the small raised area immediately anterior to root of wing) dark brown, forming a characteristic spot (it is yellow in *fusca* and var. *congolensis*). Wings darker than those of *fusca*. Abdomen dark brown.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci*. Fig. 8. Long and broad, with a fairly long, dark process at end on inner side, and a less prominent one on the outer ; a deep emargination between them ; the outer process is less evident in the dorsal (fig. 9, *b*) than in the ventral view (fig. 8). *Phallosome*. Figs. 9 ; 10. A long, broad, backwardly directed process at

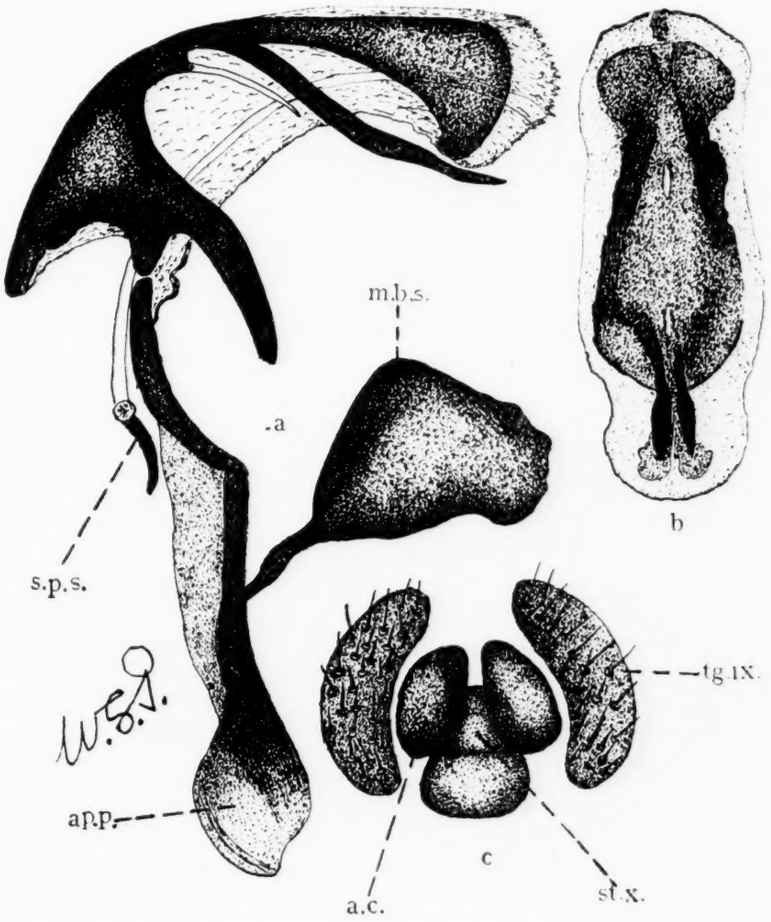


FIG. 10. *a*.—Lateral view of phallosome of *fuscipleuris* dissected out of ninth tergo-sternum showing apodeme, median binding sclerite and sperm pump sclerite ; lettering as in fig. 6, *a* ; *b*.—Signum of same ; *c*.—Terminalia of ♀ of same ; lettering as in fig. 6, *e*.

base near articulation with ninth tergo-sternum, and two processes at distal end, one very long and blade-like, the other shorter and mostly light ; the end of phallosome with a broad hood-like plate ; median binding sclerite (fig. 10, *a*) raised and somewhat triangular-shaped. ♀. The ♀ terminalia are very like those of *fusca*, but the two parts of tergum 9 are longer. The signum (fig. 10, *b*) is a very characteristic elongated plate.

DISTRIBUTION. HABITS. IMPORTANCE. *G. fuscipleuris* is mainly a Central African species but has spread eastwards. Although widely distributed in the Belgian Congo it is a rare species, and there is no doubt that it has been in the past confused with *fusca* and var. *congolensis*, which it closely resembles. At my suggestion, Dr. Schwetz has recently given a most useful synopsis of the general distribution of the various species found in the Belgian Congo, and of *fuscipleuris* he says : ' This is an extremely rare species, of which it is quite impossible to give the distribution'. It has been recorded from the Tembura and Meridi Districts of the Sudan by Bedford (1930). It has also been recorded from the Southern Masai Reserve in Kenya, from the Semliki Valley in Uganda and also from Tanganyika. The records of *fusca* Walker from Kenya, especially from the Upper Enderrit (Mara River), actually refer to this species. Lewis (1934) records collecting many specimens in the thick vegetation on the Mara River, especially in the backwaters and short gullies opening into the river, where the atmosphere was humid and where there were many well-worn game-

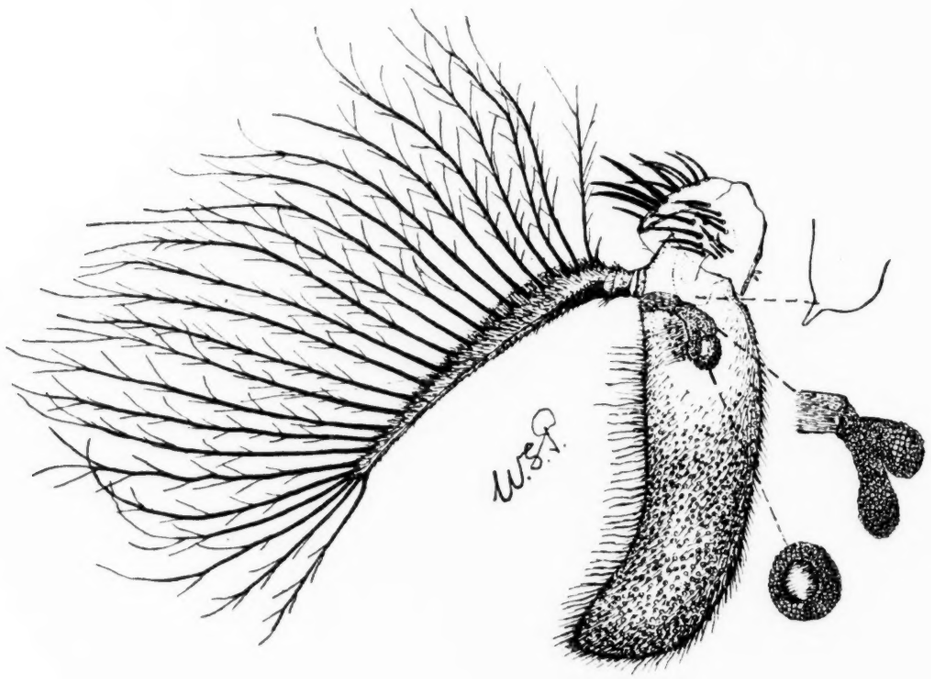


FIG. 11. Antenna of ♂ *nigrofusca* with peg and sensoria shown enlarged ; note the long hairs on the anterior border of the third segment.

paths ; it was also found at an altitude of 5,300 feet. It bites at dusk, and though mainly feeding on cattle and wild game will readily attack man. It is said by the natives to transmit disease to stock.

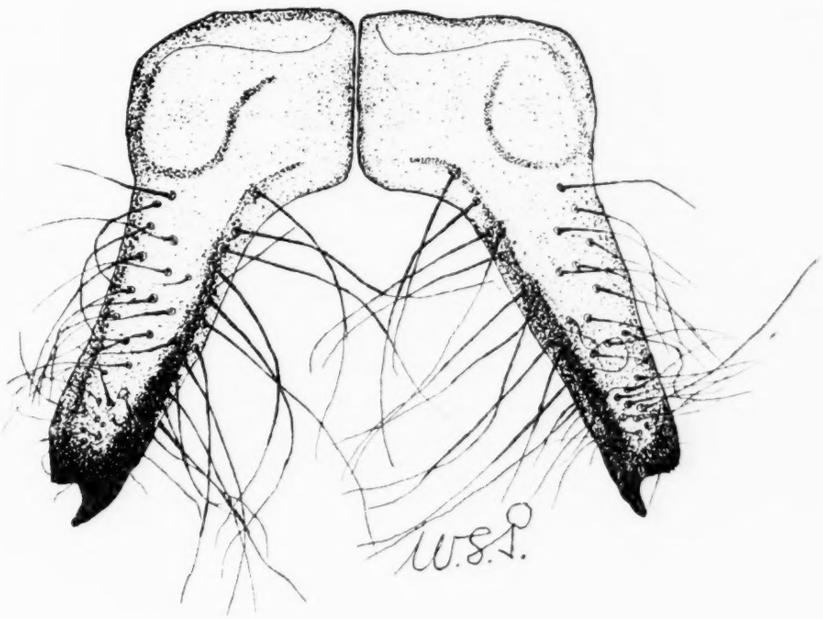


FIG. 12. Ventral view of extended anal cerci of *nigrofusca*.

Glossina nigrofusca Newstead. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A large species, measuring from 12 to 14 mm. and resembling *fusca*, but distinctly darker in colour. Antenna (fig. 11) with long hairs on anterior border of third segment and end somewhat strongly bent forwards ; palps long and slender. Thorax and abdomen dark brown.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci* (fig. 12) broad, with a stout black process on inner side, the outer side usually appearing rounded in ventral view (fig. 12) ; in dorsal view (fig. 14, *b*) the outer end shows a small nipple-like process with a deep emargination between it and the inner process. *Phallosome*. Figs. 13 ; 14. A large semi-membraneous flap at junction of phallosome with ninth tergo-sternum (fig. 13, *a*, *e* ; fig. 14, *a*) ; proximal part with a characteristic hook-like process and a separate, rather short, ventral plate ; dorsal margin of end of phallosome with characteristic folds in membrane, giving this surface in side view a serrated appearance (figs. 13 ; 14). Median binding sclerite raised and triangular-shaped. ♀. Fig. 14, *c*. The ♀ terminalia are very similar to those of *fusca* ; the signum is poorly developed.

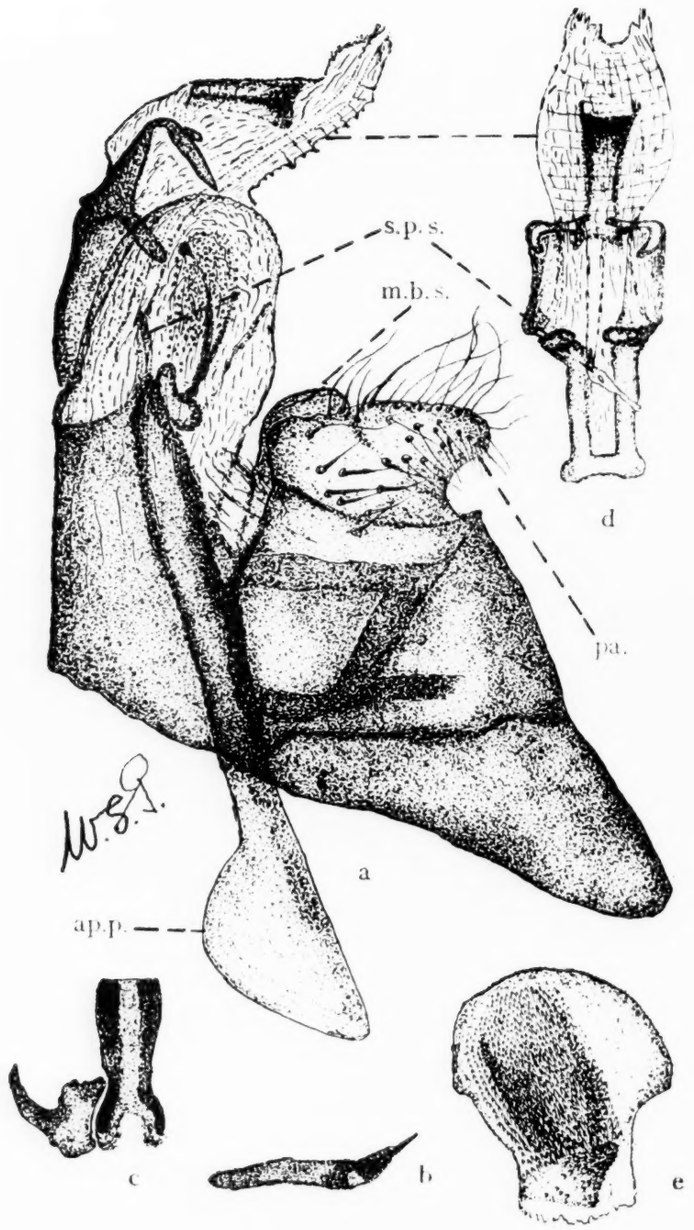


FIG. 13. *a.*—Lateral view of ninth tergo-sternum of *nigrofusca* showing phallosome embedded in its dorsal concavity; lettering as in fig. 6, *a*; *b.*—Sperm pump sclerite of same; *c.*—Basal process at distal end of phallosome; *d.*—Dorsal view of end of phallosome; note the ridges in the membrane; *e.*—Spined membraneous flap at junction of phallosome and ninth tergo-sternum.

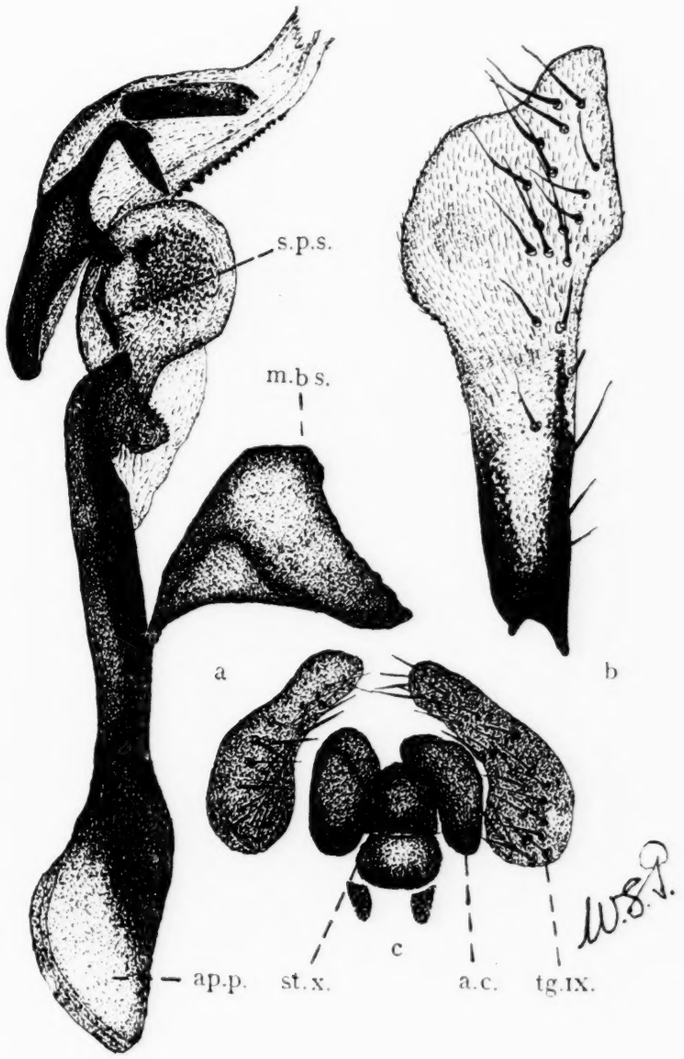


FIG. 14. *a.*—Lateral view of phallosome of *nigrofusca* dissected out of ninth tergo-sternum and showing apodeme, binding sclerite and sperm pump sclerite ; lettering as in fig. 6, *a* ; *b.*—Dorsal view of anal cerci of same ; *c.*—Terminalia of ♀ of same ; lettering as in fig. 6, *e.*

DISTRIBUTION. HABITS. IMPORTANCE. *G. nigrofusca* is a western species and is found sparsely and in scattered localities in Sierra Leone, Liberia, Ivory Coast, Gold Coast and Nigeria. It is a forest species. Austen states that it will sometimes enter houses and bite the inmates. It is not known whether this species plays any part in the spread of trypanosomes of animals.

Glossina tabaniformis Westwood. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A large species with dark wings. Antenna (fig. 15) with long aristal hairs branched almost to the end, and with rather long hairs on the anterior border of third segment, which is distinctly pointed ; palps relatively short. Wings dusky.

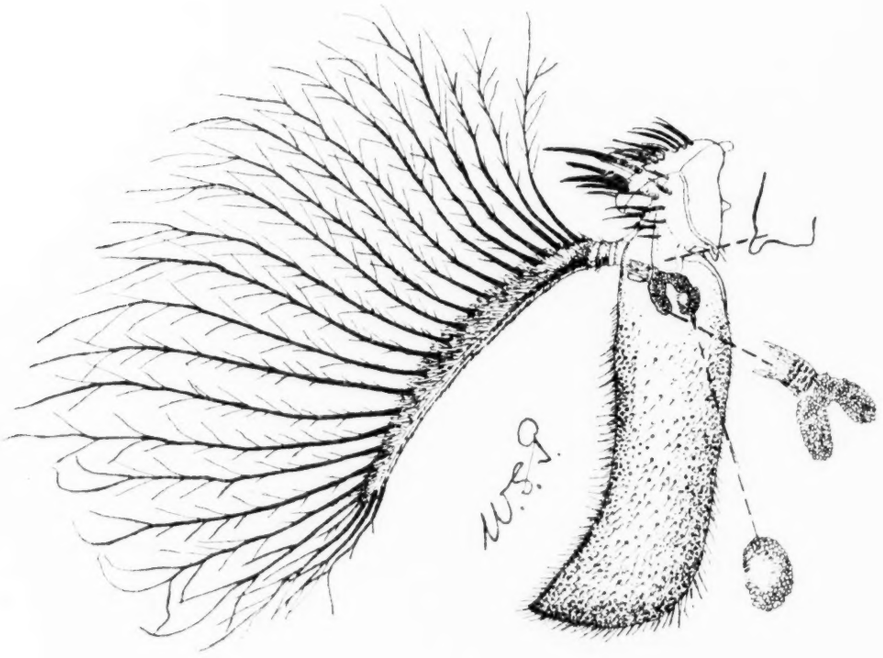


FIG. 15. Antenna of ♀ *tabaniformis* with peg and sensoria shown enlarged ; the branches of arista are long in this species.

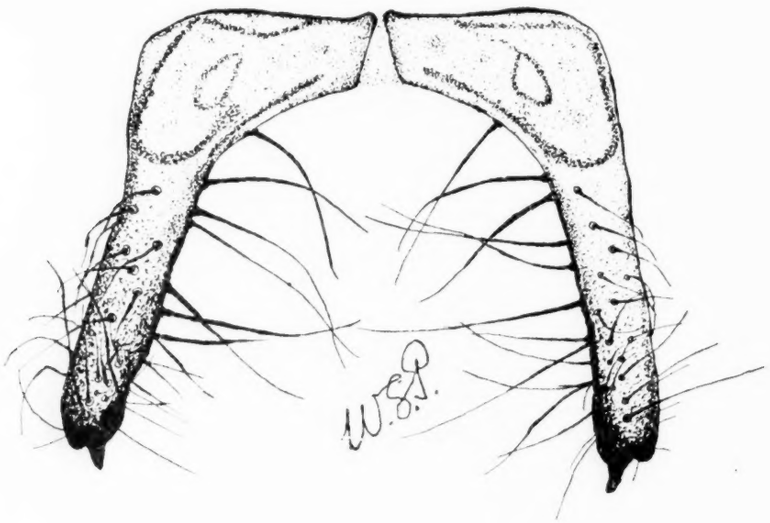


FIG. 16. Ventral view of extended anal cerci of *tabaniformis*.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Fig. 16 ; 18, *b*. Long and rather narrow, with a well-marked black process at end near inner side, and a suggestion of a process on outer side (fig. 18, *b*). *Phallosome.* Figs. 17 ; 18. Distal end with three chitinous processes, the proximal pair long and joined ventrally ; the distal process is forked at the end ; these processes

are seen from the dorsal side in fig. 17, *b*, and laterally in figs. 17, *a* ; 18, *a*. The median binding sclerite (figs. 17, *c* ; 18, *a*) is strikingly like a boot ; the paramere (fig. 17, *d*) narrows dorsally into a wide blunt projection. ♀. Fig. 18, *e*. The two parts of tergum 9 with the outer margin angled, and the plates

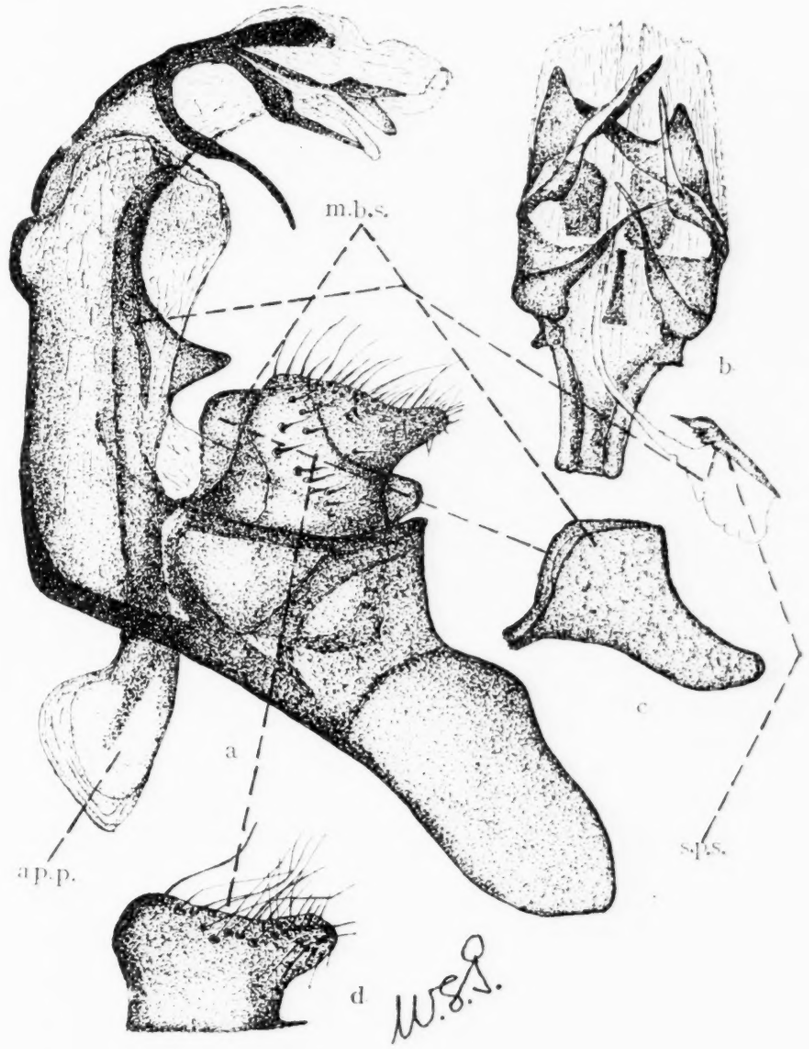


FIG. 17. *a*.—Lateral view of ninth tergo-sternum of *tabaniformis* showing phallosome embedded in its dorsal concavity ; lettering as in fig. 6, *a* ; note the characteristic boot-shaped median binding sclerite ; *b*.—Dorsal view of end of phallosome ; *c*.—The boot-shaped median binding sclerite of same ; *d*.—Paramere of same.

bent round so that their inner margins distally are closely applied (fig. 18, *e*). Signum usually somewhat lyre-shaped (fig. 17, *c*, *d*).

DISTRIBUTION. HABITS. IMPORTANCE. A West and Central African species, which has been recorded from the Ivory Coast to the Belgian Congo. Dr. Schwetz (1934), writing of the distribution of this species in the Belgian Congo, says : ' A species almost as common as the preceding (*fusca*) but its

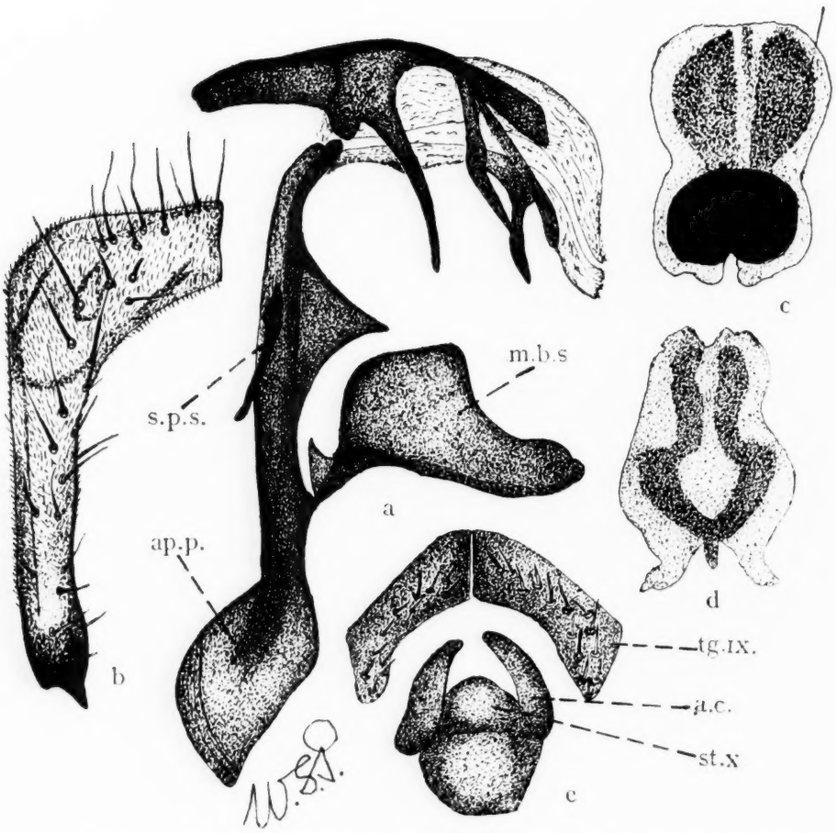


FIG. 18. *a.*—Lateral view of phallosome of *tabaniformis* dissected out of the ninth tergo-sternum and showing apodeme, median binding sclerite and sperm pump sclerite ; lettering as in fig. 6, *a* ; *b.*—Dorsal view of anal cercus of same ; *c.*—Signum of same ; *d.*—Signum of same showing variation in structure ; *e.*—Terminalia of ♀ of same ; lettering as in fig. 6, *e.*

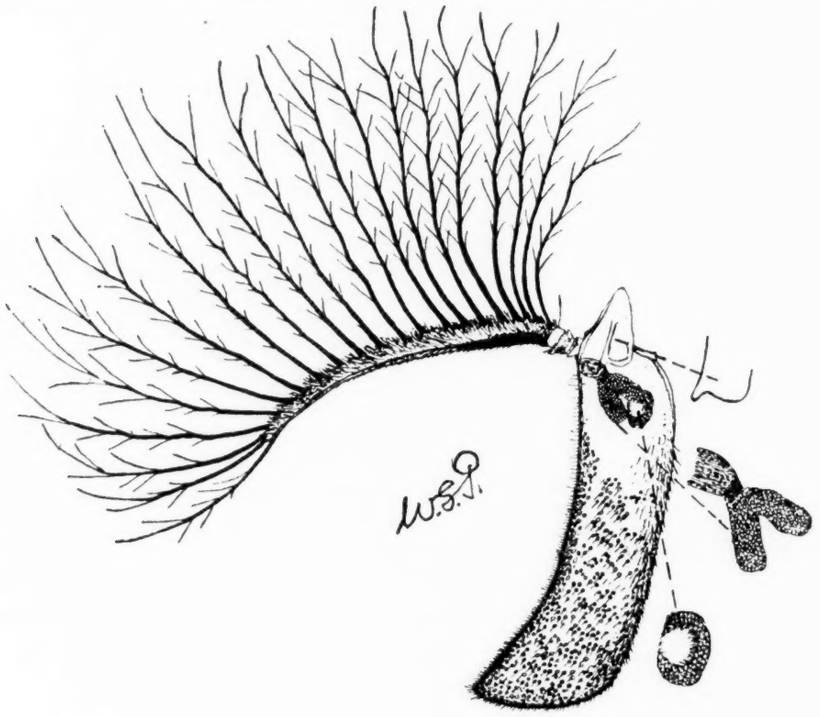


FIG. 19. Antenna of ♂ *brevipalpis* with peg and sensoria shown enlarged.

general distribution is different'; and again: 'Among the localities where *G. tabaniformis* is very common, we mention the forest of Kikwit, on the banks of the river Kwilu, from where this species spreads to the north (towards the river Kasai) and to the east (towards the river Kamtsha).' Gil Collado (1931) has recently recorded *tabaniformis* from Spanish Guinea.

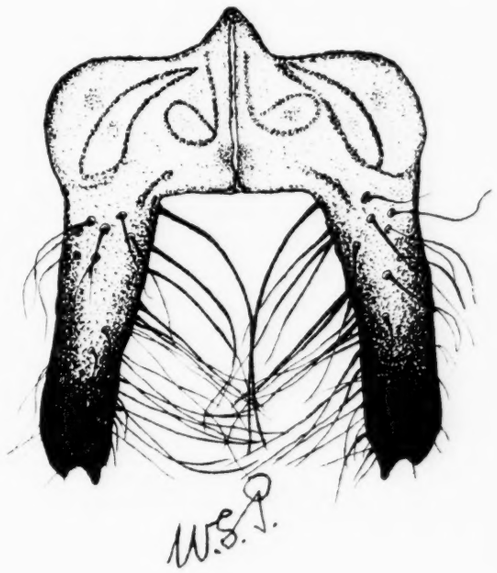


FIG. 20. Ventral view of extended anal cerci of *brevipalpis*.

Glossina brevipalpis Newstead. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A large species, with the head closely applied to the thorax. Antenna (fig. 19) with short-branched aristal hairs, the third segment pointed at end; palps very short (fig. 3, *b*), their length not greater than the width of the head.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Figs. 20; 22, *b*. Rather short and broad, with two processes at the end, that on the inner side the longer, a deep emargination between them. *Phallosome.* Figs. 21; 22. Simple, consisting of a ventral chitinous plate ending in two long bars, one on each side, their ends bending backwards (fig. 21, *b*); there is a long, finger-like, membraneous flap at junction of phallosome with ninth tergo-sternum (fig. 21, *a*, *e*); the median binding sclerite is a stout upstanding process forming a conspicuous object (figs. 21, *a*; 22, *a*). *Paramere.* Fig. 21, *a*, *c*. The paramere is small, ending in a point (rather suggesting the head of a bird, the pointed end forming the beak). ♀. Fig. 22, *c*. The two parts of tergum 9 are relatively wide, and the bristles are confined to the inner half of the plates. The signum is wanting.

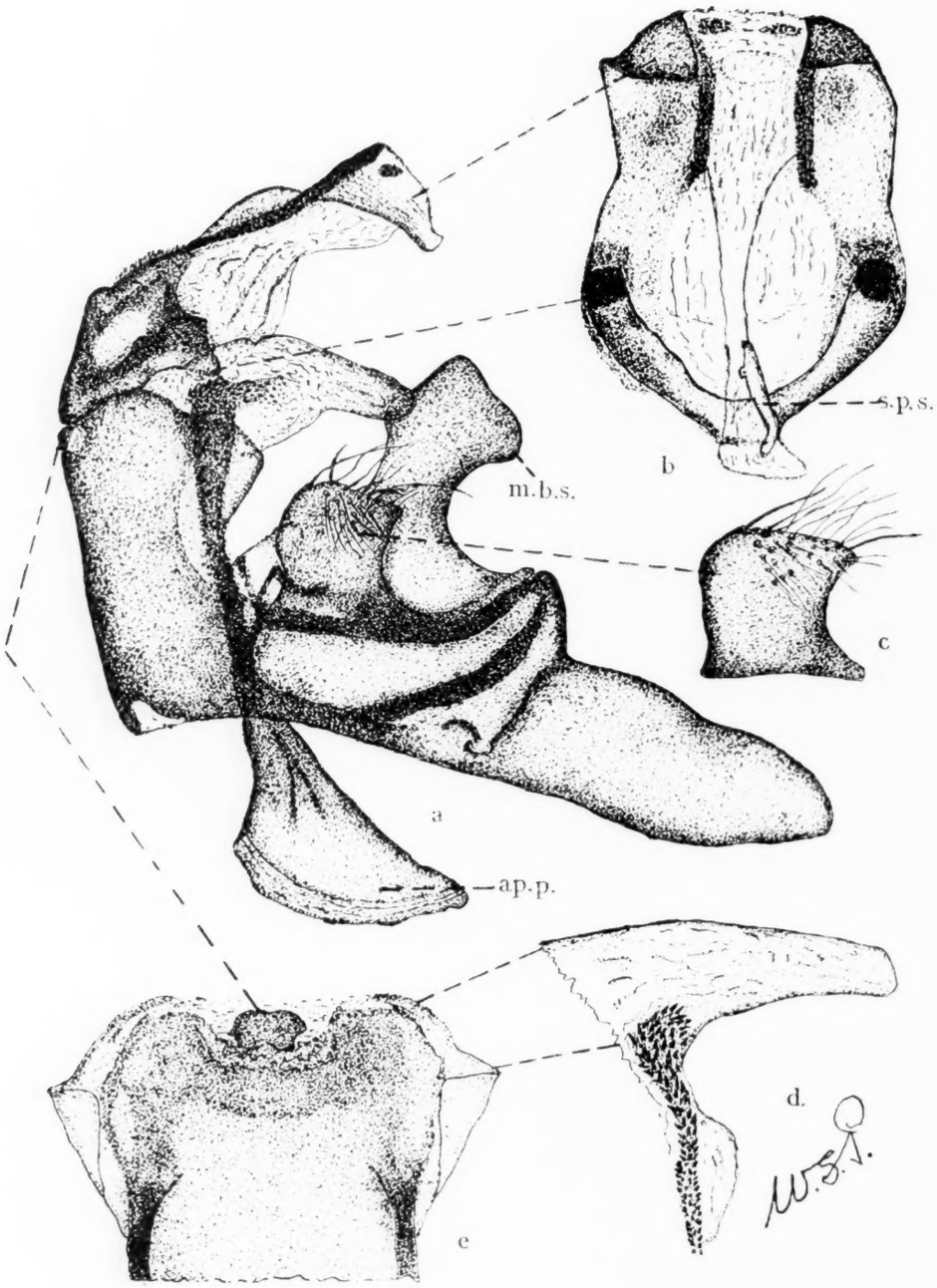


FIG. 21. *a.*—Lateral view of ninth tergo-sternum of *brevipalpis* showing phallosome embedded in its dorsal concavity ; lettering as in fig. 6, *a* ; *b.*—Dorsal view of end of phallosome of same ; *c.*—Paramere of same ; *d.*—Membraneous spined, finger-like flap of same ; *e.*—Dorsal view of end of ninth tergo-sternum showing where flap is attached.

DISTRIBUTION. HABITS. IMPORTANCE. *G. brevipalpis* is mainly an East African species. It is common along the coastal belt of Kenya, and is widely distributed in Nyasaland and Portuguese East Africa, extending into Rhodesia ; it is also common in Tanganyika, the Oriental Provinces of the Belgian Congo,

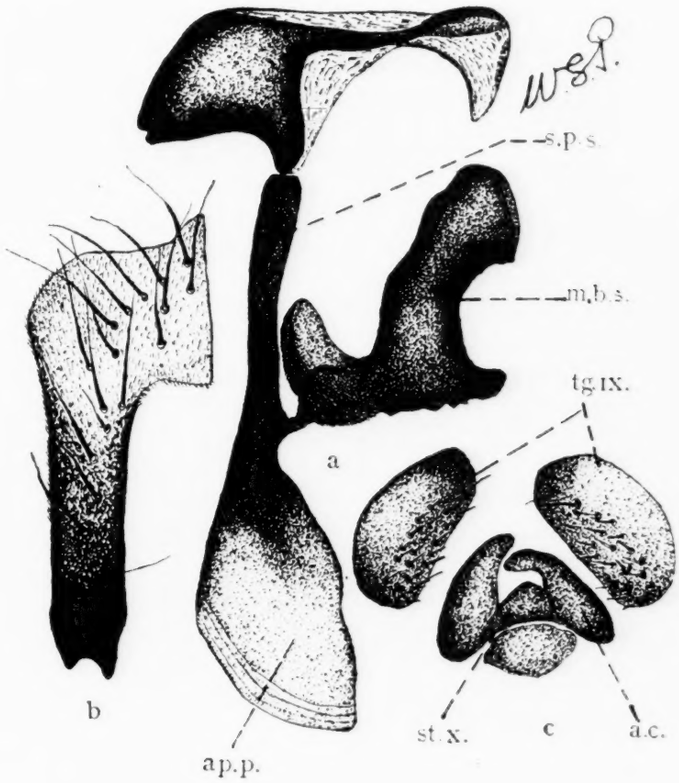


FIG. 22. *a.*—Lateral view of phallosome of *brevipalpis* dissected out of dorsal concavity of ninth tergo-sternum and showing apodeme, the characteristic raised median binding sclerite and sperm pump sclerite ; lettering as in fig. 6, *a* ; *b.*—Dorsal view of anal cercus of same ; *c.*—Terminalia of ♀ of same ; lettering as in fig. 6, *e.*

in parts of Angola and in Mozambique. Though mainly a forest species requiring shade and undergrowth, it frequents parkland and wooded savannahs. It is most active in the early morning and late in the evening. Experimentally it has been proved to be the vector of *T. brucei*, *T. congolense*, *T. simiae* and *T. caprae*.

(To be continued)

NOTES ON A LARVAL CESTODE FROM A FUR-SEAL

BY

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AND

A. J. WALKER

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In this paper the authors deal with *Phyllobothrium delphini*, a larval form from a fur-seal ; they compare it with closely related species, and offer remarks on its probable life-history and adult stage.

Phyllobothrium delphini (Bosc, 1802) Gervais, 1885

Five specimens of a large cysticercus taken from the musculature of the abdominal wall of a Falkland Islands fur-seal, *Arctocephalus australis*, were presented to us by Mr. A. W. Noel Pillers, F.R.C.V.S., F.R.S.E.

To the naked eye they all resemble a typical cysticercus, such as C. bovis or C. tenuicollis, in which the head and neck are completely evaginated ; that is, the larva consists of a bladder, continuous with a rather stout neck, terminating in a head, the whole resembling in shape a small cherry with a stalk attached (fig. 1). The size of the largest and smallest specimens was as follows :—

		Largest	*Smallest*
Bladder			
Length	..	18 mm.	12 mm.
Breadth	..	10 „	6 „
Neck			
Length	..	12 mm.	14 mm.
Breadth	..	3–4 mm.	2 „
Head			
Length	..	about 2 mm.	2 mm.
Breadth	..	„ 3 „	3 „

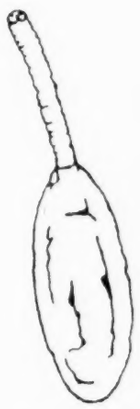


FIG. 1.—Diagram of the cysticercus entire. (× about 1½.)

Naturally the bladder varies in size within fairly wide limits, and the shape may be either almost globular or ovoid. In colour they are yellow. There is no question whatever that the larva is a simple cysticercus and not a plerocercoid. The neck is definitely flattened dorso-ventrally ; its outer surface is rugose, and bundles of longitudinal muscles arising at the junction of the bladder and the neck can be seen running along its entire length. At the base of the head the bundles first converge and then radiate fan-wise into separate bundles along the faces of the bothridia. The head varies a little in size, and is approximately 3 mm. in breadth, 2 mm. in depth (dorso-ventrally), and 2 mm. in length. When first seen it appears to bear a large number of digitate filaments, reminding one of *Parataenia elongatus*. On more careful examination, however, it is clear that the head consists of 4 membraneous bothridia with thickened margins, each folded and wrinkled exactly as is the case in *P. lactuca*, except that in our species the bothridia are not nearly so puckered and folded as they are in *P. lactuca* (fig. 2). Possibly this is due to a different distribution of the muscle fibres, or it may be that the puckerings and foldings in this immature form may become more numerous and solid in the adult worm.

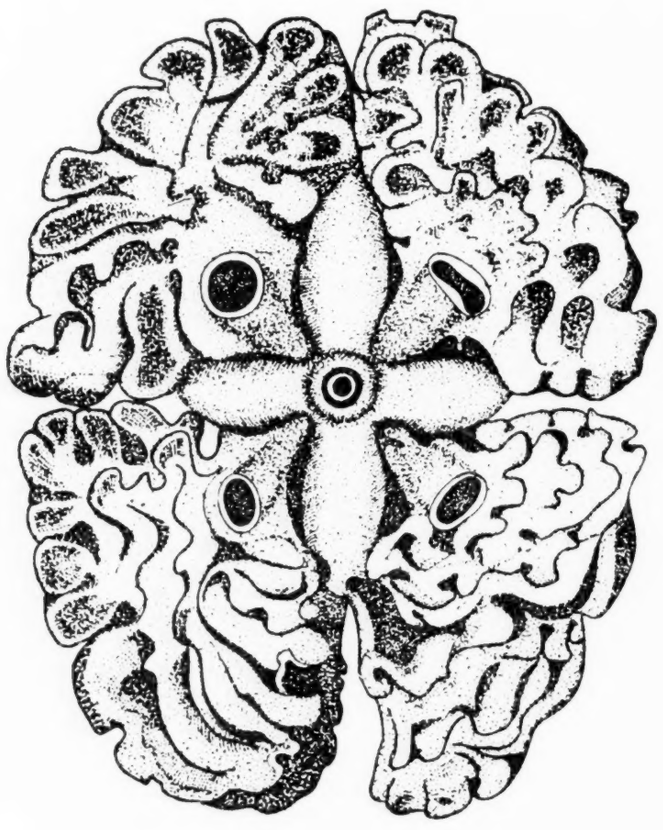


FIG. 2.—Scolex viewed anteriorly, showing suckers. (× about 30.) (D. Dagnall.)

The shape and form assumed by each bothridium varies within fairly wide limits, depending apparently on the degree of contraction. In some, each bothridium is almost wholly split into two by a deep groove running longitudinally down the centre, and reminding one of a similar condition in *P. lactuca*. Other bothridia do not show this groove, being flatter and more relaxed. Each bothridium bears a conspicuous sucker anteriorly, i.e., where it arises from the head and on its upper or anterior surface. The suckers are typical acetabula, cup-shaped, muscular, their openings being round or oval. The diameter of the suckers, including the muscular margin, varied from 200 to 350μ (fig. 2).

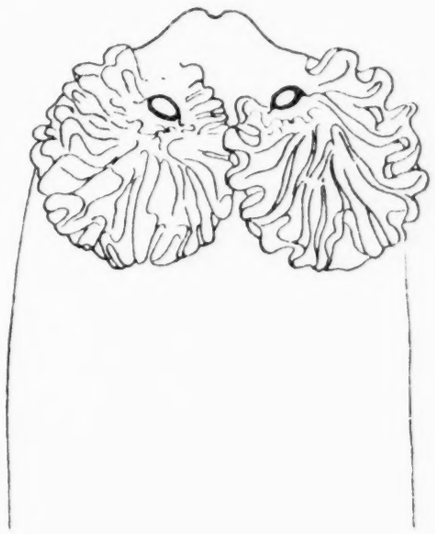


FIG. 3.—Scolex viewed laterally, showing suckers and myzorhynchus. (× about 15.) (D. Dagnall.)

In the centre of the head there is a myzorhynchus, which is so variable in size that in the examinations of our first cysticercus we concluded that this structure was absent. But in the second specimen the myzorhynchus was quite definite, broadly rounded, and had a height of about 120μ. In the centre of the myzorhynchus there is a fifth sucker, very distinct, muscular and cup-shaped, a true acetabulum. It has a diameter of from 120 to 150μ. In the specimen with the pronounced myzorhynchus this fifth accessory sucker was situated in the centre and at the apex of the myzorhynchus (figs. 3 and 4).



FIG. 4.—Diagram of myzorhynchus, showing terminal sucker, lateral view.

The following species of larval *Phyllobothriidae* have been recorded from Cetacea and the carnivorous marine mammals :—

(A). After Baylis (1932)

1. *Phyllobothrium delphini* (Bosc, 1802) Gervais, 1885.
   Synonyms *Hydatis delphini* Bosc, 1802.
   Cysticercus delphini Laennec, 1804.
   Cysticercus delphini Rud., 1810.
   Vermis delphini delphis Rud., 1810.
   Cephalocotyleum delphini delphis Diesing, 1850.
   ? *Cysticercus physeteris* Diesing, 1863.
2. *Phyllobothrium physeteris* (Diesing, 1863) Meggitt, 1924.
   Synonyms ? *Phyllobothrium delphini* (Bosc, 1802).
   Cysticercus balanae mysticeti Diesing, 1850.
   Cysticercus physeteris Diesing, 1863.
3. *Phyllobothrium inchoatum* Leidy, 1891.
   ? *P. lactuca* Van Beneden, 1850.
4. *Phyllobothrium* sp. (Carnot, 1822) Meggitt, 1924.
5. *Phyllobothrium* sp. Linton, 1905.
6. *Monorygma chamissonii* (Linton, 1905) Meggitt, 1924.
   ? *M. grimaldii* (Moniez, 1889) Meggitt, 1924.
   Synonyms *Taenia chamissonii* Linton, 1905.
   ? *Cysticercus delphini* Rud., 1819, not 1810.
7. *Monorygma delphini* (Gervais, 1870) Meggitt, 1924.
   Synonym *Stenotaenia delphini* Gervais, 1870.
8. *Monorygma grimaldii* (Moniez, 1889) Meggitt, 1924.
   Synonyms *Taenia grimaldii* Moniez, 1889.
   Cysticercus Taenia grimaldii Moniez, 1889.
   Cysticercus grimaldii Braun, 1898.
9. *Scolex delphini* Stossich, 1898.

(B). Guiart (1935) gives the following list :

Phyllobothrium delphini (Bosc, 1802), larve.
Hydatis delphinii Bosc, 1802.
Cysticercus delphini Laënnec, 1804.
Cysticercus sp. D. Bennett, 1837.
C. balaenae mysticeti Diesing, 1850.
C. physeteris Diesing, 1864.
Larve de *Phyllobothrium sp.* Ed. Van Beneden, 1868.
P. inchoatum Leidy, 1890.
Scolex delphini Stossich, 1897.
Plérocercoïde du groupe *delphinii* Baer, 1932.

Monorygma grimaldii (Moniez, 1889), larve.

Kystes vermineux de la cavité abdominale d'un Dauphin, Redi, 1684.
(*Dubium*) *delphini* Rudolphi, 1819.
Cysticercus delphini (*species dubia*) Rudolphi, 1819.
Cephalocotyleum delphini delphidis Diesing, 1850.
Phyllobothrium sp. Ed. Van Beneden, 1868.
P. delphini P. J. Van Beneden, 1870.
Stenotaenia delphini Gervais, 1870.
Cysticerque du *Taenia grimaldii* Moniez, 1889.
Cysticercus grimaldii Max Braun, 1898.
Cysticercus Taeniae grimaldii Max Braun, 1898.
Taenia chamissonii Linton, 1905.
Cysticerque du groupe *Grimaldii* Baer, 1932.

(C). Baer (1932), discussing the larval cestodes found in Cetaceans, states that the *Hydatis delphini* of Bosc is identical with *Cysticercus delphini* Rud., 1810 ; that Rudolphi in 1819 obtained from the poet-naturalist Chamisso a specimen of a cysticercus found in a dolphin, which Rudolphi named *Cysticercus delphini dubius.*

Cysticercus sp. Carnot, 1822, has never been described, and therefore no one knows what this larvae was or is ; clearly it should become a *nomen nudum.*

Some years later, Debell Bennet obtained cysticerci from a cachalot, and recorded his find to the Zoological Society, London. By an error, which has been perpetuated ever since by the majority of authors, these larvae have been described as occurring in *Balaena mysticetus* L. ; the name *Cysticercus balaena mysticetis* Diesing should be suppressed on the authority of Van Beneden, 1870. Later, Debell Bennett's specimens were named *Cysticercus physeteris* Diesing, 1863. In 1868 Edouard Van Beneden recorded from a dolphin a cysticercus having a head like a *Phyllobothrium*, which he named *P. delphini.* This was the first time that the head was noted to be that of a *Phyllobothrium.* Two years later a figure of this species was given by P. J. Van Beneden.

(D). Other records of larval cestodes from Cetacea are as follows :

In 1870, Gervais found a larva in a dolphin, which he named *Stenotaenia delphini.*

Moniez in 1889 found in a dolphin a larva which he named *Taenia grimaldii.*

Leidy in 1891 recorded a larva from *Mesoplodon bidens*, which he named *P. inchoatum.*

Stossich, 1898, found two kinds of cestode larvae in *Grampus griseus* Cuvier, viz. :

a. Cysticercus delphini.

b. Scolex delphini. In this form there are 4 simple bothridia, each with a sucker, and there is a terminal sucker in the middle of the head.

The figure given by Stossich of the head of this larval form resembles very closely that given by Zschokke (1888) of *Tetrobothrium longicolle* (= *Anthobothrium longicolle* Molin, 1858), except that in the latter species there is no fifth accessory sucker. It also bears some resemblance to *Monorygma elegans* Monticelli, 1890, save that in the latter species each bothridium bears an accessory sucker at its anterior extremity, whereas in Stossich's larva the accessory sucker is situated near the middle of each bothridium. We would call attention to the reemblance of *Scolex delphini* Stossich, 1898, to *Scolex polymorphus* of Rudolphi, 1819.

Linton (1905) recorded *Taenia chamissonii* from *Lagenorhynchus acutus* Gray, which he thought identical with *C. delphini* Rud., 1819, not 1810. Linton's species was a cysticercus, apparently identical with *C. grimaldii* Moniez, 1889. Linton also recorded another species which he named *Phyllobothrium* sp., the identity of which is uncertain.

Phyllobothrium sp. Rennie and Reid, 1912 ; from the blubber of *Ogmorhinus weddelli*. The authors state that in this larval form the head ' consists of four much pleated or folded bothria. Accessory suckers are absent.' In the absence of accessory suckers this form differs from *P. delphini* (Bosc, 1802).

Plerocercoides portieri Guiart, 1935. This is a plerocercoid measuring only 5 mm. in length.

It will be obvious from the lists given by Baylis, Guiart and Baer that the synonymy of what is now generally known as *Phyllobothrium delphini* is most involved, and it is perhaps impossible to say with any degree of certainty what the larvae were which the various early authors obtained. In many instances the description given of a larva was quite inadequate, and its specific characters cannot now be determined.

But it is abundantly evident that the following definite species of larvae are known from Cetacea and *Phocidae*, viz. :—

1. *P. delphini* (Bosc, 1802) Gervais, 1885. This is a large cysticercus, identical, in our opinion, with the species which we describe in this paper.

2. *P. chamassonii* Linton, 1905 (*P. grimaldii*, Moniez, 1899). This is a cysticercus in which there is a larva with an extremely long filamentous hair-like neck, bearing at its extremity a comparatively small head. It is quite different from *P. delphini* (Bosc, 1802).

3. *Scolex delphini* Stossich, 1898. This does not appear to differ from what is generally known and accepted as *Scolex polymorphus*.

4. *Plerocercoides portieri* Guiart, 1935. This larval form is a very small plerocercus and not a cysticercus.

5. The species which we describe in this paper and which we consider to be identical with *P. delphini* (Bosc, 1802).

The larvae referred to under 2, 3 and 4 are totally unlike the one we record. Fuhrmann (1931), discussing the morphology of Tetraphyllodian scoleces, figures a head obtained from a leopard-seal which closely resembles that in our species.

Our species differs from *P. delphini* only in possessing an accessory sucker which is situated at the apex of a myzorhynchus of very variable size.

Probable Life-History

P. J. Van Beneden, in 1876 (pp. 207–8), wrote as follows :—

' Do the cetacea generally live on fish and do they become the prey of some aquatic carnivora ? . . . There have been seen in the substance of the muscles of many species or rather in the layer of blubber which covers the skin, agamous cestode worms of the genus *Phyllobothrium*, which can only accomplish their evolution in some large *squalus*. There must be contests between dolphins and sharks, contests in which the dolphins are worsted in spite of their superiority. These Phyllobothria have been found in *Delphinus delphis*, the *Tursio* and the *Ziphius*. As the *Orca* attacks the whale and feeds upon its flesh there would be nothing surprising in our finding in these large cetacea some agamous cestode destined to pass through the last phase of its evolution in this terrible carnivorous mammal.'

Sharks can and do attack and eat portions of cetaceans and seals, which for a variety of reasons may have become incapable of defending themselves ; and it is known that the cysticerci found in seals are capable of retaining their viability for at least 11 days after the death of the host.

Baer calls attention to the fact that no other instance is known in which cysticerci occurring in a warm-blooded mammal become adult in a cold-blooded animal.

There are, of course, instances in which the reverse is the case.

The hypothesis that the *C. delphini* (Bosc, 1802) found in the blubber and musculature of Cetacea and *Phocidae* actually has its adult stage in *Carchariidae* is up to the present merely a matter of conjecture.

The present authors have made an extensive search of the literature in an endeavour to ascertain whether any adult Phyllobothriids, *sensu lato*, have a scolex comparable with that of *P. delphini* as figured by P. J. Van Beneden in 1870.

He shows a scolex with four sessile, oval, rather shallow bothridia, having thickened, convoluted, wrinkled margins, and each bearing anteriorly (i.e., near its point of attachment) a single acetabulum. The centre of the head is occupied by a small flattened eminence which does *not* bear an accessory sucker, and no fifth sucker is mentioned by subsequent authors.

Linton, in 1890, describes a species of Tetraphyllid obtained from *Trygon centrura*, to which he gave the name *Phyllobothrium foliatum*. Linton stated that in his specimen the four bothridia were thin, leaf-like, sessile, and each bothridium was bordered by a distinct row of loculi with crenulate margins. The edges of the bothridia were more or less ruffled and folded, each bothridium being provided with a single supplemental disc on its anterior margin.

His species was from a ray, and one of us obtained the same species on the Ceylon Pearl Bank, also from a ray (*Rhynchobatus djeddensis*). We cannot, however, identify *P. delphini* Van Beneden with *P. foliatum* of Linton, not because of any marked morphological differences between the two, but primarily because we are unable to understand how it would be possible for a ray to eat the flesh

of either Cetacea or *Phocidae*. We would remark upon the improbability of any species of ray being concerned in the life-history of the cysticercus of seals and whales, on the ground that their teeth are in the form of flat plates, resembling the stones forming a pavement, and are clearly designed to crush and not to tear. Their food consists largely of *Molluscs* and *Arthropods*.

Furthermore, in 1922, Linton described a new cestode from the man-eater and mackerel sharks (*Carcharodon carcharias* and *Isurus dekayi*), which he named *Phyllobothrium tumidum*. The head of this worm is very similar to that of *P. foliatum*, each bothrium being frilled and puckered ' to such a degree that their real nature is difficult to interpret,' and each bearing a conspicuous accessory sucker. The centre of the head is occupied by a dome-shaped myzorhynchus, which does *not*, however, bear a fifth sucker. He further points out that Leidy, in 1887, described under the name *Taenia loliginis* (which name Leidy changed later to *Phyllobothrium loliginis*) a cysticercus from a squid, which bears a close resemblance to Van Beneden's figure of *P. delphini*.

Linton reports the presence of *P. loliginis* from numerous other hosts, including *Raja laevis*, *Raja ocellata*, *Mustellus canis*, besides certain squids, such as *Ommastrephes illecebrosa* and *Loligo pealii* ; and he concludes by saying : ' Evidence which points to *Phyllobothrium tumidum* as the probable adult stage of *P. loliginis* is based on the similarity in form and details of structure of the scolex. In making this comparison the terminal sucker of *P. loliginis* is interpreted to be an evanescent larval structure as in *Scolex polymorphus*.

' The feature which presents the greatest difficulty is that part of the scolex which is posterior to the bothria. In *P. loliginis* there is a rather conspicuous neck portion, which is sharply marked off from the strobile proper. In *P. tumidulum** the strobile appears to begin close to the bothria, although segments do not make their appearance at once. In the structure of the bothria and auxiliary suckers, and in the axial portion of the scolex there is close agreement.'

Neither Baer (1932) nor Guiart (1935) refer either to Linton's suggestion or to his paper, and one can only assume that these authors had not seen it.

Linton clearly suggests that *P. loliginis* is identical with *P. delphini*, and that these cysticerci, which, as noted above, occur in squids, various rays, seals, porpoises, dolphins and whales, have their adult stages in *Carcharodon carcharias* and *Isurus dekayi*, the man-eating and mackerel sharks respectively.

With reference to the feature which presented difficulty to Linton in his assumption that the head of *P. tumidum* in the man-eating and mackerel sharks and the head of *P. delphini* (= *P. loliginis*) in squids and rays were the same, it occurs to us that in the development of the larva into the adult form segmentation would begin immediately behind the head, and that the ' neck ' portion would atrophy with the bladder as the strobila develops in the spiral valve of the shark.

We would, however, call special attention to Linton's remark that ' In

* This evidently is a *lapsus calami* for *P. tumidum*.

making this assumption the terminal sucker of *P. loliginis* is interpreted to be an evanescent larval structure as in *Scolex polymorphus*.'

We have examined five specimens of *P. delphini* from the Falkland Islands seal (*Arctocephalus australis*), and through the kindness of Dr. S. W. Kemp, F.R.S., and the ' Discovery ' Committee we have been permitted to examine specimens of cysticerci identical with ours obtained from the blubber of (*a*) the leopard-seal, *Hydrarga leptonyx*, and (*b*) *Otaria bryonia*, Falkland Islands, ' Discovery ' Expedition, 1931, J. E. Hamilton.

In all these cysticerci the apex of the myzorhynchus was occupied by a terminal sucker, a true acetabulum, which is definitely absent in the adult worm *P. tumidum*. Hence arises the necessity for Linton's assumption.

It is, we believe, true that the head or scolex of a cysticercus becomes, and, in fact, actually is, the head of the adult worm, and that no changes whatever in its morphology take place from the time it is fully developed in the cysticercus stage to the time when, attached to its final host, it has budded off a chain of segments.

In the case of a *Scolex polymorphus*, however, the situation is quite different. *Scolex polymorphus* is the name given to a series of larval forms : some have simple bothridia without accessory suckers, but with a terminal sucker in the centre of the head ; others have the bothridia divided transversely into four loculi, the anterior loculus in each becoming the accessory sucker which may, or may not, persist. In addition, there is again a terminal sucker in the centre of the head. All stages between these two extreme types are known, and it is believed that various genera are represented. The fully-grown larvae of the various species included under *Scolex polymorphus* have not been determined. Once the larva is fully grown, there appears to be no proof that any morphological changes take place in the head whilst the strobila is developing in the final host. It may be that a central terminal sucker present in a half-grown *S. polymorphus* of any particular genus may disappear in the fully-grown larva ; and it would even seem probable that the progressive diminution in the size of the terminal sucker, the older the larva becomes, may mean that the acetabulum actually ceases to exist when the larva is fully grown. We know of no case, however, amongst cysticerci in which the head of the larval form is not identical in structure with the head of the adult worm. But it may well be that our knowledge is incomplete and that the terminal sucker in *Cysticercus delphini* actually atrophies during the development of the larva in the cysticercus stage.

In this connection it is interesting, if not important, to note that, whilst up to the present no terminal fifth sucker has been noted on the myzorhynchus of *P. delphini*, we found a small one on the scolex of our specimens.

Although the specimens of this species, lent to us by Dr. Kemp, were very distinctly larger than ours, the terminal sucker was definitely smaller. We were accordingly compelled to entertain the possibility of the terminal (fifth) sucker gradually disappearing as the larva becomes fully grown. We do not, however, believe that atrophy of the sucker takes place during the development of the larva into an adult worm in the final host ; so that in this larval form it would

appear that a terminal (fifth) accessory sucker is present in young cysticerci, but that, when the larva has grown to its full size, the terminal sucker has entirely disappeared.

We agree with Linton that the larval form, known as *Phyllobothrium delphini* (Bosc, 1802) = *Phyllobothrium loliginis* (Leidy, 1887), found in seals, dolphins, whales and squids, becomes adult in the man-eating and mackerel sharks, and that the adult worm is *Phyllobothrium tumidum* Linton, 1922.

No other adult *Phyllobothrium* is known having a scolex like that found in *Cysticercus delphini* (Bosc, 1802). If our conclusion is correct, then the name *Phyllobothrium tumidum* Linton, 1922, would become a synonym of *Phyllobothrium delphini* (Bosc, 1802) Gervais, 1885.

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THE TRANSMISSION OF PARALYTIC RABIES IN TRINIDAD BY THE VAMPIRE BAT (*DESMODUS ROTUNDUS MURINUS* WAGNER, 1840)

BY

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In previous publications, Hurst and Pawan (1931, 1932) described an outbreak of acute spreading myelitis in the island of Trinidad, caused by the virus of rabies and affecting 17 human beings and 450 animals. Neither the origin nor mode of transmission had then been definitely determined, but the opinion was tentatively expressed that the vampire, or blood-lapping, bat was the vector. The disease made its first appearance in animals in 1925 and in human beings in 1929, and up to the present over 2,000 animals and 53 human beings have died from the condition. No case of recovery has been observed. Though there is a large number of stray, ownerless dogs, no canine or other carnivore has so far been shown to be affected. The disease assumed the paralytic form in both man and animals (two of the latter are, however, reported to have shown a tendency to bite), and in no instance did transmission from one animal to another occur by the bite of any of these 2,000 animals. Close association in the stalls and the ingestion of fomites, presumably contaminated, failed to cause infection. A striking feature of the human cases—and this has invariably been the earliest manifestation of the disease—was the development and localization of subjective symptoms of paraesthesia followed by paresis and paralysis in the foot or toe—though in two later cases the arm was first involved. This symptom was a very definite characteristic, even in the first 13 cases which occurred in the year 1925, and, as the victims belonged to the labouring classes who walk with bare feet, the possibility of the entrance of the infection through a wound on the sole of the foot was considered. Dead frogs are often seen lying on the roads, and may be trampled upon by the bare feet ; but histological examination of about 120 frogs collected from infected areas, together with the inoculation into rabbits of the brains of 20, failed to reveal the presence of Negri bodies or the virus of rabies.

Early in 1931, owners of live-stock in certain parts of the island where the disease was occurring in animals observed that their animals were being unduly bitten by the vampire (blood-lapping) bats, and the lay opinion was expressed that the vampire bat was in some way or other responsible for the disease. Vampire bats abound throughout the island ; they are nocturnal in their habits, and during the day congregate in caves, hollows of trees, subterranean channels, and beneath bridges, etc. (Pl. IV, fig. 1). They emerge at night to feed on blood, and in certain districts human beings and animals are invariably bitten, unless artificial lighting is employed to ward them off. This definitely nocturnal

habit on the part of these bats is known to all classes of the community. But in 1931 the unusual spectacle was observed in infected areas of bats flying about and attempting to bite animals, and even man, during the day in bright sunshine.

BATS AS VECTORS OF DISEASE

Three species of vampire bats (the only known species) inhabit Central and South America. Their attacks upon man and warm-blooded animals and their blood-lapping propensities had been observed by the earliest writers. The *Encyclopaedia Britannica* (1911) quotes Peter Martyr as saying, soon after the conquest of South America by the Spaniards, that ' in the Isthmus of Darien there were bats which sucked the blood of men and cattle when asleep to such a degree as to even kill them.' On landing in South America the first Spanish settlers were informed by the natives that the destruction and absence of horses were due to the attacks of blood-lapping bats, though geological evidence suggests an earlier disappearance. Missionaries and colonists soon introduced horses and cattle from Europe ; but Condomine (quoted in the *Encyclopaedia Britannica*), writing in the 18th century, recorded that in Borja (Ecuador) and in other places the cattle thus introduced were exterminated by bats. With our present knowledge of the habits of the vampire bat, and of the rate and extent of blood regeneration after haemorrhages in man and animals, it seems not unlikely that the heavy losses, amounting almost to extinction, of cattle and horses in such wide territories were due, not so much to the actual ' sucking ' or lapping of blood by bats and the coincidental depletion, as to the development of some disease by their bites.

Stiles and Nolan (1931), in the key-catalogue of parasites reported for Chiroptera (bats), with their possible public health importance, say : ' The habits of bats are so totally distinct from those of man that, as far as known, bats do not play much of a rôle in connection with the diseases of man,' though it is added that ' African sleeping sickness of man is experimentally transmissible to at least three bats.' Wenyon (1926) does not mention Chiroptera as harbouring pathogenic parasites transmissible to man. And Lyon (1931) reports that bats have never been shown, up to 1931, to be ' carriers of any infection ' to man. Kumm (1932), however, found that mechanical transmission of yellow fever virus by the bite of the vampire bat is possible under experimental conditions, but adds that this mode of infection ' is probably of little or no epidemiological importance.' Dunn (1932*a*) demonstrated in Panama that fruit- and insect-eating bats could be successfully infected with the horse trypanosome, *Trypanosoma hippicum* Darling, but that owing to the short and fatal course which the disease ran in them, together with the fact that they do not feed on man or animal, concluded that ' they cannot become very important carriers or reservoirs of the disease in a community.' In further experiments (1932*b*), however, he

showed that this same equine trypanosomiasis (' murrina ') of Panama could be transferred to the vampire bat (*Desmodus rotundus*), and that infection could last as long as 27 days. He was of the opinion that under natural conditions equines would acquire the disease through the bite of these ' vampires.' Later, Clark and Dunn (1932) found 18·6 per cent. of the bats in Panama infected with a trypanosome morphologically similar to *T. cruzi*, the cause of Chagas' disease in human beings.

In 1908 a severe epizootic appeared among the horses, mules and cattle in the State of Santa Catharina and the neighbouring districts of South Brazil, destroying over 4,000 bovines and 1,000 equines, and erroneously described as ' mal de caderas de bovinos.' Carini (1911), in São Paulo, by the finding of Negri bodies in affected animals and the results of animal inoculation, diagnosed the condition as rabies, though he added that he failed to detect Negri bodies in the first rabbit passage of material in which they were present. He further noticed that canine rabies was comparatively rare in infected areas, and suggested that the disease was being transmitted by some wild animal. He emphasized the fact that the inhabitants had observed the unusual phenomenon of bats flying about and actually biting animals in daylight, and that animals thus bitten subsequently died. In his own words, ' the number of rabid dogs seen in affected districts did not appear to bear relation to that of rabid cattle and horses ; this fact points to the possibility of the infection being transmitted by wild animals. Thus at St. Pedro d'Alcantara many trustworthy persons assured us that they had seen bats attacking cattle on pastures in broad daylight and biting them. Bovines thus bitten all died some time afterwards. It would have been interesting to verify experimentally whether these bats were suffering from rabies or were carriers of infection ; unfortunately we were unable to obtain the bats for this purpose.' The outbreak continued to spread uninterruptedly up to 1916 and 1920 among the cattle and horses of Paraguay, Uruguay, Argentine and Brazil, leaving human beings totally, and dogs almost entirely, unaffected.

With the acceptance of the diagnosis of rabies, a rigorous and extensive destruction of dogs was enforced : in six months of the year 1912 over 6,799 of them were killed. Importation into affected areas of all canines was also strictly forbidden, but the epidemic nevertheless continued to spread among cattle, horses and mules. It was further observed that the incidence of the disease was greatest in the vicinity of forests, and that rivers offered no barrier to the spread of the disease. The suspicion was therefore again raised that some flying sylvan animal was responsible for the spread of the epidemic.

Haupt and Rehaag (1921) studied the disease in the municipality of Blumenau and the neighbouring districts of South Brazil, and definitely confirmed Carini's diagnosis that the condition was rabies. Other workers—e.g., De Souza (1926, 1927), in Rio Grande do Sul and Matto Grosso, Brazil ; Kipshagen (1930) ; Rosenbusch (1930), in Paraguay ; Costa (1931), Quiroga and others (1931),

in Corrientes in the Argentine—also came to a similar conclusion. Haupt and Rehaag remarked particularly on the disparity between the very few cases of canine rabies and the heavy mortality from the disease, and observed that the losses were greatest in the vicinity of thinly populated forests, while streams impassable to dogs offered no barrier to the spread of the disease. Furthermore, the destruction of dogs did not lead to a diminution in the number of cases. Their attention was also drawn to the unusual phenomenon of bats flying about during the day, shrieking and fighting with one another. ' Such abnormal aggressiveness and biting fits must surely be regarded as signs of disease,' was their final opinion.

The inhabitants of that district were also convinced that vampire bats were spreading the disease, and specimens brought to Haupt and Rehaag were identified as *Phyllostoma superciliatum* Burmeister (1854). They were ' nose-leaf ' bats, but in the stomach of two partly digested blood was present. On July 1st, 1914, a ' nose-leaf ' bat was found biting an 8-days-old calf which, since birth, had been kept in a bat-proof stable. Twenty-seven days later, though protected from further bites, the calf developed typical paralytic rabies. Experimental study of the brain of the bat was unfortunately not undertaken. In 1916 Rehaag succeeded in producing paralytic rabies in a rabbit and a guinea-pig with an emulsion of the medulla of a *Phyllostoma superciliatum* bat which had been biting a bovine, and in sections of the rabbit and the guinea-pig Negri bodies were found. On the strength of this evidence, Haupt and Rehaag (1921) concluded that the epidemic of paralytic rabies which ravaged the live-stock of South Brazil from 1913 to 1918 was spread by bats. The satisfactory results of protection of animals against their bites subsequently confirmed this opinion. De Souza (1926), however, failed to infect guinea-pigs and rabbits by allowing *Phyllostoma* bats to feed on them, and concluded, on insufficient evidence, that in Florianopolis, Brazil, the disease was not spread by bats. Quiroga, Acosta and their co-workers (1931) quote Parreiras Horta as having also shown from experimental work on rabbits and guinea-pigs that bats (*Phyllostoma*) are not the transmitting agent. On the other hand, Kraus and Duran (1932*a*), working in Chili with brain material of a rabbit (forwarded to them from Trinidad) which had died after intracerebral inoculation with the brain of a suspected bat, obtained the typical symptoms of rabies with Negri bodies in experimental animals, and agreed with the opinion previously expressed by Carini, Haupt and Rehaag, and Hurst and Pawan that the paralytic rabies of South America and Trinidad was being spread by certain species of bats.

Lima (1934), in Matto Grosso, undertook a series of investigations in connection with the spread of paralytic rabies in Brazil, to ascertain the possibility of infecting vampire bats under experimental conditions with the virus, and to determine the course of infection in these mammals. He was able to demonstrate that the blood-lapping *Desmodus*, when inoculated artificially with bovine rabies, can infect another bat, and that a bat thus infected can transmit

the disease to cattle within 1 to 5 months later. Further, an infected bat, capable of transmitting disease, may appear quite healthy. Later, at Santa Catharina he was able to produce rabies experimentally in rabbits by the inoculation of brain material from 2 bats found in infected areas. Lima concluded that herbivorous rabies is carried by haematophagous Chiropteras which form the Desmodontidae family, especially by the *Desmodus rotundus* Geoffroy (*D. rufus* Wied), which is the most prevalent species.

THE VAMPIRE BAT AND RABIES IN TRINIDAD

Technique

(*a*) *Animal Inoculation.* The brain and spinal cord of the bat or experimental animal were removed as soon as possible after death and preserved in sterile glycerine and saline at a temperature of 5–10° C. until required for use. An emulsion, approximately 10 per cent., of the cerebrum, cerebellum, medulla or cord was made in normal saline, and 0·2 c.cm. and 0·1 c.cm. were employed for subdural inoculation of rabbits and guinea-pigs respectively, and 2·0 c.cm. for intracerebral inoculation of the larger animals in the left parieto-occipital area or in the cerebrum near the middle line. For intra-ocular inoculation, the conjunctiva was anaesthetized with cocaine and 2–3 drops were injected into the anterior chamber. Post-mortem examination was directed especially to the detection of abscess formation or sepsis, and was performed within 6 hours after death ; otherwise the animal was slaughtered while moribund so as to avoid decomposition, which takes place very rapidly in this climate. The monkeys employed were *Macacus rhesus*, imported from India and kept in cages on the laboratory grounds in the city of Port-of-Spain, where natural infection could not take place. The calves and dogs were obtained from districts where the disease did not exist. Except when otherwise stated, inoculation was by the subdural or intracerebral route.

(*b*) *Examination for Negri Bodies.* (1) *Smear preparations* of the hippocampus. These were used only for the routine examination of bats caught in the field in large numbers. Giemsa, Van Gieson, Gerlach, and Muromzew's borax methylene blue methods were employed. The last two were the most satisfactory. (2) *Sections.* In the case of bats one entire cerebral lobe was fixed, except when acetone was used as a fixative. For other animals a portion of the hippocampus, cerebellum or optic nucleus (noyau optique basal), as suggested by Nicolau et al. (1933), was fixed in Zenker, Bouin, sublimate alcohol with or without formol, or Henke and Teller's acetone. As stains, Mann, Stutzer, Giemsa as modified by Levaditi, were employed. With the acetone fixative, Lentz's and Schonwetter's methods were used. For routine diagnosis Mann's method was the most satisfactory ; for detection of the internal structure Stutzer's was employed ; and for rapid diagnosis Lentz's.

1. Examination of Bats

In the literature available, Hayman (1932) lists 31 species of bats recorded for Trinidad. There are fruit-, insect- and fish-eaters, etc., and only one, a blood-' sucker ' or blood-lapper (as the bat does not suck but laps blood), viz. *Desmodus rufus* Wied, 1826, for which Ditmars and Greenhall (1935), in their study of the taxonomy of the family, suggests the specific name of *Desmodus rotundus murinus* Wagner, 1840. (Pl. IV, fig. 4.)

From August 1st to September 8th, 1931, 157 bats were examined in smear preparations, stained by Van Gieson and Gerlach methods, for the presence of Negri bodies in the hippocampus, and 35 others were sectioned and stained by Mann's. No attempt was made at differentiating the species of bats. They all, however, proved negative. In addition, 52 other fruit-eaters (*Artibeus planirostris trinitatis*, cf. Pl. IV, fig. 3) caught during the day were pooled in various lots and injected intracerebrally into 6 rabbits. None of them developed rabies.

On September 10th, 1931, a ' nose-leaf ' bat was caught flying in a chemist's shop at 11.05 a.m. in Sangre Grande village. It was sent to the laboratory, the brain was immediately removed, and one portion was fixed in Zenker and another injected intracerebrally into a rabbit on September 11th. Sections of the hippocampus stained by Mann's method showed Negri bodies, the rabbit developed paralysis of the hind legs on the 23rd, and died on the following day, i.e., in 13 days. The hippocampus fixed in Zenker and stained by Mann also showed Negri bodies. This was the first conclusive demonstration of the infection of Trinidad bats with the virus of rabies. Subsequent examination of this bat proved it to be *Artibeus planirostris trinitatis*, a fruit-eater.

On September 14th, 1931, two bats were observed screeching and fighting with each other about 10 a.m. over the heads of a group of men standing in an open space in Tacarigua village, where cases of paralytic rabies were occurring in animals. They both fell to the ground in a close embrace and were killed and brought to the laboratory. Unfortunately the species was not determined. The brains of both were removed and injected intracerebrally into a rabbit on the 15th. On the 25th, the rabbit showed emaciation with paralysis of the left side of the body, and death took place on the 26th, i.e., in 11 days. Examination of the hippocampus fixed in Zenker and stained by Mann's method showed Negri bodies.

It was then decided to concentrate attention on the examination by animal inoculation of bats exhibiting abnormal habits, such as flying or attempting to bite animals during the day, or fighting with one another. It has been possible up to date to detect infection in this manner in 22 bats caught in various parts of the island. The details of the animal experimentation on some of them are given here.

1. *Bat 20* (*Desmodus*) was seen flying low in a cattle-pen at Waterloo Dairy, Couva, at about 5 p.m. on March 23rd, 1932, and was knocked down with a

hat by an attendant. On April 5th, injection was made into 2 rabbits. On the 16th they were both seen to be thin. On the 18th, at 8 a.m., one rabbit was weak, but there was no evidence of paralysis. At 9 a.m. the front legs showed definite paresis; at 10 a.m. they were paralysed, and the hind legs showed paresis. At 11 a.m. both front and hind legs and the hind quarters were paralysed. Death took place on the 19th, i.e., in 14 days. Negri bodies were present in hippocampus.

The other rabbit showed paresis of the front extremities on the 19th, with complete paralysis of all four legs on the 20th. Death took place the same day, i.e., in 15 days. Negri bodies were present in the hippocampus. From this animal further inoculation was made subcutaneously into 2 rabbits on the 21st. On May 7th there was paralysis of the front legs and the rabbit was emaciated. Towards the afternoon the paralysis involved the hind legs, and death took place on the 8th, i.e., in 17 days. Negri bodies (few) were present in the hippocampus. The other animal also developed paralysis and died on May 11th, i.e., in 20 days. Negri bodies were not seen. From this, inoculation was made on the 12th into 4 guinea-pigs, 2 intracerebrally and 2 subcutaneously. Death took place in 14, 10, 28 and 32 days, and doubtful Negri bodies were seen.

From the rabbit which died on April 19th inoculation was made on April 21st into 2 other rabbits, one intracerebrally and the other subcutaneously. The former died with paralysis in 27 days and the latter died on May 10th, i.e., in 19 days. Negri bodies were present in both instances. From the latter, injection was again made on June 28th into another rabbit. Emaciation with paralysis followed and death occurred on July 18th, i.e., in 20 days.

2. *Bat 10* (*Desmodus*) was seen attempting to bite a cow at Exchange Estate, Couva, at 10 a.m. on March 24th, 1932. It was killed with a stick and brought to the laboratory, where the brain and cord were at once removed. On April 5th, 12 days later, 2 rabbits were injected, and, at various periods subsequently, 18 other rabbits. The results are given in Table I. In most instances paralysis was seen to develop in one or other extremity. The variations in the intervals between the inoculations and death both here and in Tables II and III may be explained by the fact that the rabbits were not of uniform size, and the brains were preserved in glycerine saline in periods varying from 1 to 27 days at a temperature of 5–10° C.

From rabbit 5, 1 c.cm. was injected intracerebrally into 2 dogs on June 16th, 1932. On July 2nd, 16 days later, one dog (female) was seen to be off her feed. The following day she was apathetic and indifferent, though she had formerly been very playful and active. She remained in this state until the 6th, when she was once more bright and playful and quite well. She remained well for 10 months, after which she was destroyed. No Negri bodies were seen.

The other dog (a male) went off his feed on July 16th, i.e., 4 weeks after infection. The following day he looked ill and would not eat, and there was

TABLE I

Rabbit no.	Inoculation made from	Intervals in days between injection and death	Mode of inoculation	Negri bodies
1	Bat	14	Subdural	+
2	,,	15	,,	+
3	Rabbit 1	16	Subcutaneous	+
4	,, 1	20	,,	+
5	,, 3	13	Subdural	+
6	,, 5	16	,,	—
7	,, 5	21	,,	+
8	,, 7	15	,,	—
9	,, 3	18	,,	—
10	,, 3	20	,,	—
11	,, 5	11	,,	+
12	,, 6	12	,,	—
13	,, 6	15	Subcutaneous	+
14	,, 5	17	Subdural	—
15	,, 5	17	Subcutaneous	+
16	,, 5	30	Intra-ocular	+
17	,, 5	26	Subdural	+
18	,, 2	15	,,	—
19	,, 3	22	,,	—
20	,, 5	25	Subcutaneous	—

paralysis of the hind legs. On the 18th, all four legs were paralysed and he could not rise. There was also profuse salivation on that day. On the morning of the 19th he was found dead. Post-mortem examination showed congestion of the brain and softening of the cord. Negri bodies were present in optic nuclei and hippocampus.

On the following day, July 20th, 2 other dogs were injected with material from this male dog. One died the following day from traumatic cerebral haemorrhage ; the other remained well until August 31st, i.e., $5\frac{1}{2}$ weeks later, when paralysis of the hind legs with profuse salivation appeared and continued for 2 days. Death took place on September 3rd. ' Doubtful ' Negri bodies seen.

3. *A white bat* (*Diclidurus albus*), a non-' blood-sucker ' (a rare species), was caught at 11 a.m. on May 10th, 1932, resting on a cow in Carapichaima village, where paralytic rabies was occurring in animals. On the 12th, inoculation was made into 2 rabbits and 4 guinea-pigs, 2 intracerebrally and 2 subcutaneously. On June 8th one rabbit showed weakness and paralysis, and death took place the following day, i.e., in 28 days. The other survived 32 days. Negri bodies were found in both cases.

The two guinea-pigs injected intracerebrally died on June 1st and 2nd respectively. One showed Negri bodies. The other two injected subcutaneously

showed much emaciation, developed paralysis on June 13th, and died on the 14th and 15th respectively. One showed Negri bodies, the other was doubtful.

4. *Bat S. 1* (*Desmodus*) was found fighting with another bat in Caura village, and attempting to bite cattle during the day. It was caught and forwarded by a sanitary inspector on July 22nd, 1932. The brain and cord were immediately removed, and on August 8th were injected into a rabbit. There was rapid emaciation, and on the morning of August 14th it was found dead. A few Negri bodies were seen. On September 13th injection was made into another rabbit. There was progressive emaciation, with paresis of the right hind leg on October 3rd, i.e., in 20 days. On the following morning both hind legs, and in the afternoon all four legs, were paralysed, and death took place on October 6th, i.e., in 23 days. This rabbit showed the unusual feature of attempting to bite the attendant before paralysis developed. From this animal another rabbit was inoculated *intra-ocularly* on November 19th. Extreme emaciation followed with paralysis, and death occurred on December 15th, i.e., in 26 days. Doubtful Negri bodies were present in hippocampus.

5. *Bat 13* (*Desmodus*) was received from the District Medical Officer, Mayaro, on February 27th, 1933, with a history of flying about and attempting to bite animals during the day. Inoculation was made into a rabbit on March 11th. Emaciation was rapid, paralysis appeared on the 24th, and death took place on the 25th, i.e., in 14 days. From this rabbit inoculation was made into 3 other rabbits on March 31st. Emaciation with paralysis followed, and death took place in 9, 12 and 18 days respectively. Negri bodies were found in 2 animals. From the rabbit which had died on March 25th, inoculation was made on June 26th with brain material preserved in glycerine saline for 3 months. The rabbit showed emaciation with paralysis, and death occurred on July 22nd, i.e., in 26 days. This was again carried into another rabbit on the 24th. Emaciation and paralysis developed, and death occurred on September 12th. No Negri bodies were found in the hippocampus.

On March 11th, *intra-ocular* inoculation was made into a rabbit with the brain of bat 13. There was much emaciation and weakness with paralysis on the 28th, and death took place on the 29th, i.e., in 18 days. Negri bodies were present in the hippocampus.

6. *Bat 14* (*Desmodus*) was caught biting a cow at 7 a.m. on August 8th, 1933, in Cunupia village, where paralytic rabies was occurring in animals. The cow had been vaccinated 5 weeks previously with anti-rabies vaccine. The brain and cord of the bat were immediately removed, and on the following day, August 9th, were injected into a rabbit. There was rapid emaciation, and on the 15th there was paralysis of both hind legs. Death took place the following day, i.e., in 7 days. Brain sections showed Negri bodies. A portion of the rabbit's brain was injected on August 27th into another rabbit, and paralysis with death followed 9 days later. A few Negri bodies were present.

Another portion of the brain and cord of the same bat 14 was also injected,

but *subcutaneously*, on August 9th into a calf. On the 26th there was weakness of the hind legs, with difficulty in rising from the ground. Two days later the animal showed emaciation, and a keratitis had developed. When urged to walk, it would sway from side to side, roll the hind quarters, and progress forward with a staggering gait. On September 2nd the staggering was less marked, but on the 4th the keratitis, emaciation, weakness, rolling of the hind quarters, and staggering were all much more evident, and continued to increase until October 14th, when paralysis of both fore and hind quarters and profuse salivation, with much grinding of the teeth, developed—all the symptoms typical of paralytic rabies as it occurs under natural conditions in the field. Death took place the following day, i.e., 9 weeks after infection. Post-mortem examination showed the lesions characteristic of the disease, i.e., congested brain, soft cord, and haemorrhages in the heart muscle. Both the hippocampus and cerebellum showed Negri bodies. With the brain of this calf, inoculation was made into a rabbit on November 1st. Death with paralysis and emaciation followed in 13 days, and Negri bodies were present in the brain.

7. *Bat 16* (*Desmodus*) was received from Mr. 'M.', of Pointe-à-Pierre, on August 18th, 1933, with the following history: 'This morning my house boy was bitten on the foot by a bat which remained in the house and continued flying from room to room. During my attempts to drive it out it flew straight at both myself and wife, thereafter settling on the wall about 10 ft. from the floor. When my wife passed under it, it again flew directly at her with its mouth wide open. I might add that whilst it was hanging on the wall it kept turning its head from side to side with its mouth wide open.'

On the 19th, a rabbit was injected intracerebrally. Paresis of the legs with emaciation and a definite keratitis appeared on September 3rd, with paralysis on the 4th and death on the following day, i.e., in 17 days. Negri bodies were found in the hippocampus.

The boy was given a course of anti-rabies vaccine from the 18th, and has remained well.

8. *Bat 15* (*Desmodus*). A sanitary inspector submitted this bat with the following history: 'The bat was seen to fly around 4 men who were standing near a mule pen on Exchange Estate, Couva, at about 8.30 a.m. on August 14th, 1933. Afterwards it flew among the animals in the pen and attempted to bite a cow, when it was chased away by the men, caught, and killed. Paralytic rabies is occurring among animals in the district.'

On the 19th, a rabbit was injected. On September 3rd it was emaciated and the hind legs showed paresis. On the 4th there was paralysis, and death took place on the 5th, i.e., in 16 days. Negri bodies present. From this, 2 other rabbits were injected on September 14th, one intracerebrally (r.1) and the other intra-ocularly (r.2). In both cases there was rapid emaciation, and death took place on the 18th, i.e., in 4 days. 'Doubtful' Negri bodies present. From rabbit r.2 *further intra-ocular* injection was made into another rabbit on the

19th. Emaciation was obvious in 48 hours. Paresis of the left legs and the left side of the body appeared on the 23rd, paralysis of all 4 legs on the 24th, and death occurred on same day, i.e., in 5 days. Negri bodies were present, but few.

From rabbit r.1, which had died on the 18th, further injections were made into 7 more rabbits, as shown in Table II.

TABLE II

Rabbit no.	Date of injection	Date of death	Interval between injection and death	Negri bodies
1	19. 8.33	3. 9.33	15 days	+
2	19. 9.33	9.10.33	20 ,,	+
3	1.11.33	19.11.33	18 ,,	—
4	20.11.33	3.12.33	13 ,,	—
5	8.12.33	21.12.33	13 ,,	—
6	30.12.33	25. 1.34	26 ,,	?
7	27. 3.34	13. 4.34	17 ,,	—

9. *Bat 21* (*Desmodus*) was found biting a cow at Malabar village, Arima, at 7 a.m. on May 8th, 1934. Though repeatedly chased away it returned to attack the cow, and was killed with a stick in the act of biting. Paralytic rabies existed among animals in the district. The brain and cord and salivary glands were dissected out and preserved separately, and on the following day 2 c.cm. of an emulsion of the brain and cord were injected intracerebrally and 2 c.cm. subcutaneously into the cheek of a *Macacus rhesus* monkey. On the 17th, 8 days later, the monkey was screaming continuously and throwing himself into a violent rage whenever anyone approached the cage. He would bite at the bars, and lacerate his mouth and face in consequence. On the following day he was salivating profusely and was unable to eat or swallow. Death took place the same day, i.e., in 9 days. Post-mortem examination showed congestion of the brain and much softening of the spinal cord—in some parts the latter was as soft as butter. Negri bodies were present in the hippocampus, cerebellum and optic nuclei.

Inoculation into calf and dog. Two c.cm. of an emulsion of the medulla of this monkey were injected intracerebrally, on May 18th, into 2 calves, and on the 25th into a dog. Five days later *calf no. 1* seemed unduly quiet, but was grinding its teeth continuously. There was an anxious expression in the eyes, and there were alternate backward and forward nervous movements of the ears, with repeated licking of the lips—evidence of commencing salivation. On the 25th, these manifestations were more marked, especially the grinding of the teeth, and salivation became excessive. On the 27th, it was knocking its face and mouth against the wall, causing laceration and bleeding. On the 28th, there

was paralysis of the left front leg and quarters, and the animal was unable to rise from the ground. Death took place on the 30th, i.e., in 12 days. Negri bodies were seen in the hippocampus and cerebellum.

Calf no. 2, injected on the same day and with the same quantity, showed symptoms on the 23rd, i.e., 5 days later, when profuse salivation began and a well-marked paralysis of hind legs appeared. On the 24th, he was unable to rise, and death took place on the 26th, i.e., in 8 days. Negri bodies were present in the hippocampus and cerebellum.

Calf no. 3. From calf no. 2, another calf was inoculated on July 7th. Profuse salivation, excessive grinding of the teeth and paralysis of the hind quarters developed on the 11th, and death took place on the 12th, i.e., in 5 days. Negri bodies were present, but few.

Calf no. 4. From calf no. 3, another calf was injected on July 19th. The same symptoms as with calf no. 3 appeared in 4 days, and death took place on the 5th day. ' Doubtful ' Negri bodies were seen in hippocampus.

Dog. One white ' frizzy ' dog was injected on May 25th with 2 c.cm. intracerebrally, and 1 c.cm. subcutaneously into the cheek, of an emulsion from the monkey. Eight days later, June 2nd, he was howling frequently without any obvious cause. On the 4th, the howling was continuous and loud. On the 6th, he was paralysed in both hind legs ; and death took place the same day, i.e., in 12 days. Post-mortem examination showed congestion of the brain and softening of the cord, with Negri bodies in large numbers in the hippocampus and cerebellum.

Rabbit. On May 9th, a rabbit also was injected with the brain of bat 21. There was paralysis of the left side on the 21st, with death on the 22nd, i.e., in 13 days. Negri bodies of a small size were found in the hippocampus and optic nuclei. This strain was carried on into 7 more rabbits, as shown in Table III.

TABLE III

Rabbit no.	Date of injection	Date of death with paralysis	Days between inoculation and death	Negri bodies
1	9. 5.34	22. 5.34	13	+
2	26. 5.34	16. 6.34	21	+
3	10. 7.34	20. 7.34	10	—
4	28. 7.34	12. 8.34	15	+
5	16. 8.34	22. 8.34	6	—
6	5. 9.34	10. 9.34	5	—
7	12. 9.34	30. 9.34	18	+
8	3.10.34	24.10.34	21	—

The salivary glands of the same bat 21 were dissected out on May 9th, 1934, and 2 c.cm. were injected intracerebrally and 2 c.cm. subcutaneously into the cheek of a *Macacus rhesus* monkey. Eleven days later the monkey was violent, was screaming continuously and biting fiercely at the cage, and had lost much flesh. On the 24th, he would hide himself in a corner of the cage, as if frightened, but would still scream loudly and bite at the bars of the cage. The following day the screaming ceased, but there was paresis of both upper extremities and the upper half of the body. Later in the day the upper half of the body was bent forward, and he was in a helpless, moribund condition. Death took place on the 27th, i.e., in 18 days. Post-mortem examination showed softening of the brain and cord, with Negri bodies in both cerebellum and hippocampus.

10. *Bat 4290* (*Desmodus*) was caught in Guanapo village on October 20th, 1934, and was kept in captivity with blood feeds until March 30th, 1935 (i.e., for nearly 5½ months), when the brain was removed and injected subcutaneously into the cheek of a guinea-pig. On April 29th, it was seen to be paralysed on the left side, and death took place on the 30th, i.e., in 4 weeks. Negri bodies were present. From this, another guinea-pig was again inoculated in the cheek on the same day, the 30th. Death with paralysis took place on May 20th, i.e., in 20 days, and the brain showed Negri bodies (few).

11. The brains of 2 other bats caught on April 10th, 1935, in St. Joseph village, attempting to bite animals during the day, were injected on April 30th subcutaneously into the cheek of 2 guinea-pigs. They produced paralysis with death in 20 and 21 days. Negri bodies were found in one guinea-pig and not in the other.

The brains of 10 other bats (*Desmodus*) showing abnormal habits, but not examined histologically, were also injected into rabbits and produced paralysis with death in periods varying from 8 to 21 days ; Negri bodies were found in the hippocampus. The further passage was not undertaken. In addition, however, the brains of about 16 others (*Desmodus*) caught flying during the day, and not showing Negri bodies in their brains, were inoculated intracerebrally into rabbits, and produced rapid emaciation with paralysis, death occurring in periods varying from 5 to 12 days ; but careful examination failed to reveal the presence of Negri bodies in the rabbits' brains. Such specimens were labelled as free from rabies. The results yielded by them will be discussed later, in comparison with Carini's failure to detect Negri bodies in experimental rabbits, and with Remlinger's emphasis on the value of cross-immunity tests in the identification of rabid and other neurotropic viruses.

2. Cross-Immunity Tests

The subcutaneous inoculation of Semple's phenol-killed vaccine preserved in 0·5 per cent. carbolic sometimes produces in rabbits after 4–5 injections rapid and progressive emaciation with death. Recourse was therefore had to

ether-killed vaccine prepared by Remlinger's method. A series of 18 rabbits were given daily subcutaneous injection with 1–2 c.cm. of a 4 per cent. ether-killed brain emulsion of *virus fixe*, and 8 others with bat virus for 3 weeks ; and 21 days later blood was drawn from those immunized with *virus fixe* for the serum neutralization tests, while for the protection tests an infecting dose of the live virus was given 23 days after the last prophylactic inoculation. The rabbits employed were of approximately the same size and weighed about 1,800 to 2,000 gm. After death they were not examined for the presence of Negri bodies. The results are here tabulated.

1. *Rabbits immunized with virus fixe, then inoculated with bat virus XXI*

Intracerebrally 0·2 *c.cm. of* 0·5 *per cent.*	*Subcutaneously* 1 *c.cm. of a* 5 *per cent.*	*Intra-ocularly* 2 *drops of a* 5 *per cent.*
0	0	0
0	0	+ 35
0	0	0
+ 21	0	0
0	0	0
0	0	0

\+ = paralysis with death and period in days between inoculation and death.
0 = animal remained alive and well for 2 months.

2. *Rabbits immunized with bat virus XXI, then inoculated with virus fixe*

Intracerebrally 0·2 *c.cm. of* 0·5 *per cent.*	*Subcutaneously* 1 *c.cm. of a* 5 *per cent.*
+ 14	+ 20
+ 10	0
0	0
+ 18	+ 29

Controls

Four rabbits were inoculated intracerebrally with the live *virus fixe* and 4 with the bat strain. Death took place in every instance, as follows :—

Virus fixe	*Bat virus*
+ 9	+ 13
+ 8	+ 10
+ 10	+ 12
+ 9	+ 9

These results show protection by *virus fixe* against infection with bat virus, but only feeble protection by the latter against the former. It may be added that similar results were obtained in comparing *virus fixe* with a bovine strain, undertaken to determine from which strain vaccine should be prepared for prophylactic use on animals exposed to infection in the field.

3. Serum Neutralization Tests

One c.cm. of antiserum collected from the rabbits immunized with *virus fixe* as mentioned above was carefully mixed with a 1 per cent. of the living virus in varying quantities, and was left at room temperature, 27° C., for 2 hours; 0·2 c.cm. were then injected intracerebrally into each of 30 rabbits, with the following results :—

Antiserum + 1 c.cm. *virus fixe*	0	0	+ 10	+ 9	+ 8	+ 9
Antiserum + 0·5 c.cm. *virus fixe*	0	0	+ 12	+ 9	+ 10	0
Antiserum + 0·2 c.cm. *virus fixe*	0	0	0	0	0	0
Antiserum + 0·5 c.cm. bat virus	+ 15	0	0	+ 12	+ 19	+ 16
Antiserum + 0·2 c.cm. bat virus	0	0	0	0	0	0

Controls

1 c.cm. normal serum + 0·2 c.cm. *virus fixe* =	+ 10	+ 9	+ 8	+ 10
1 c.cm. normal serum + 0·2 c.cm. bat virus =	+ 14	+ 16	+ 16	+ 20

These results show the neutralizing effect of *virus fixe* antiserum towards *virus fixe* and bat virus.

4. Epidemiological Evidence Based on Personal and Clinical Histories

Reference has been made to the development of subjective symptoms in one or other limb as the earliest manifestation of the disease in human beings. In the labouring population of Trinidad, hysterical neuroses play little or no part in their struggle for existence, and the tragic sequelae to a history of a ' burning sensation and pain ' in an extremity subsequent to a bat-bite have been constant and true. In every instance but one (case no. 31, ' C.B.'), where inquiry was instituted, the localization of these symptoms coincided with the site of a bat-bite ; and up to the present date every case (except no. 39) volunteered the information or admitted that he had been bitten by a bat at some time prior to the development of symptoms. Table IV shows this relationship.

TABLE IV

Year	No.	Name	Interval between bite and first symptom	Approximate date and situation of bat-bite	History
1929	1	C.F.	—	—	Paralysis of right leg, July 12th
	2	M.H.	—	—	Weakness of left leg, July 21st
	3	V.L.	—	—	Inability to move limbs, July 20th
	4	E.	—	—	Weakness of both legs, July 22nd
	5	J.C.	—	—	Weakness of both legs, July 30th
	6	K.W.	—	—	On July 27th, awoke in his sleep with a burning pain in sole of left foot. Pain went up the leg in the morning, and during the day he could not raise the leg
	7	Y.B.	—	—	Fever for some days, then paralysis of both legs, Aug. 7th
	8	L.F.	—	—	Weakness of left leg, Aug. 9th
	9	J.G.	—	—	While at work in the forest, felt a tingling and numbness in the left leg. Towards the night the leg became weak and he could not raise it
	10	W.M.	—	—	Pain and numbness in right big toe, Aug. 10th, then weakness extending to leg
	11	D.C.	—	—	Fever followed by weakness of both limbs
	12	S.	—	—	Fever followed by pains in both legs
1930	13	A.B.	—	—	Fever, retention of urine, followed by paralysis in both legs
	14	J.G.	—	—	Burning sensation in sole of left foot, Aug. 2nd, then weakness of left leg
	15	A.P.	—	—	Burning sensation with pain in left foot, Aug. 16th
	16	A.G.	—	—	Tingling and weakness in both legs, Oct. 16th
1931	17	A.A.	—	—	Fever followed by pain, Jan. 22nd, in right leg, then weakness
	18	E.G.	4 weeks	July 6th, on 2nd toe, left foot	Aug. 2nd, fever, followed by loss of sensation and power to move left leg, with pains and cramps
	19	F.K.	3 weeks	About Sept. 11th, on left big toe	Weakness and heaviness in both legs on Oct. 2nd
1932	20	D.G.	3 weeks	Last week, Dec., 1931, situation not known	Weakness and pain in right leg, Jan. 21st

TABLE IV—(*Continued*)

Year	No.	Name	Interval between bite and first symptom	Approximate date and situation of bat-bite	History
1932	21	B.M.	6 weeks	2nd toe, right foot and forehead, Jan. 29th	Mar. 14th, numbness in right foot
	22	B.	6 months	Oct., 1931	Weakness and coldness in left hand and arm on Mar. 10th
	23	P.	3 weeks	Feb. 24th, on right elbow	Pain in *right elbow*, Mar. 16th, then right shoulder with dysphagia
	23*a*	Child of case No. 23	2 weeks	Same night, Feb. 24th, and in same room but on *right foot*	Paralysis of right foot
	24	A.	11 months	Dec., 1931, on both feet	Pain and weakness in both lower extremities, Nov. 1st
1933	Two cases of acute transverse myelitis, but virus of rabies not detected by histological or experimental examination				
1934	25	S.	3½ months ago	April, on toes, right foot	Pain on left leg and pains in joints, Aug. 19th
	26	A.J.	6½ months	Feb., on toe, left foot	Tingling on left leg and pains in joints, Aug. 19th
	27	A.P.	3½ weeks ago	July 25th, left big toe	Weakness on both legs, Aug. 11th
	28	J.M.	5 weeks	3 weeks, June, both big toes	Pain and tingling left leg, July 26th
	29	J.B.	?	Patient unconscious ; no history obtainable	Inability to use both legs, Jan. 19th
	30	A.	4 weeks	Feb. 14th, on right little finger	Pain in left wrist and forearm and weakness in grasp in left hand, Mar. 14th
	31	C.B.	3 weeks	Feb. 12th, toe on *right foot*	Fever and paralysis on right *upper extremity*, Mar. 2nd
1935	32	N.M.	6 weeks	Feb. 8th, on right big toe	Fever, followed by pain and weakness in left leg, Mar. 21st
	33	V.J.	3 weeks	Mar. 10th, on left 2nd toe	Burning sensation and weakness in left leg, April 1st
	34	C.C.	5 weeks	Last week, Feb., on 3rd toe, left foot	Pains in toe and leg during night of Apl. 4th
	35	A.C.	4 weeks	Mar. 4th, on both big toes	Cramps, burning sensation and pain in left leg, Apl. 4th ; and in right leg, Apl. 5th
	36	V.Jo.	4 weeks	Mar. 12th, on left little toe	Fever, followed by burning sensation and pain in left foot, Apl. 12th
	37	J.S.	4–5 weeks	1–2 weeks, on right big and 2nd toes	Burning sensation in sole of left foot with weakness of left leg, Apl. 12th

TABLE IV—(*Continued*)

Year	No.	Name	Interval between bite and first symptom	Approximate date and situation of bat-bite	History
1935	38	R.	6 weeks	Mar. 11th, on both big toes and 2nd toe, left foot	Itching and numbness with pain in right leg, Apl. 26th
	39	T.O.	?	On foot	1. Bat bite 20 years ago 2. Never been bitten within last 20 years
	40	M.H.	3 weeks	On Apl. 29th, right index finger	Pain in right arm and difficulty in swallowing, May 20th
	41	N.S.	4 weeks	Apl. 23rd, on left shoulder	Burning sensation in shoulder and back, difficulty in swallowing, May 23rd
	42	W.G.	6 weeks	Mar. 13th, on 3 toes, left foot	Pain in left leg from toes to knee, May 24th
	43	D.P.	7 months	Last week, Oct., 1934, 2nd toe, left foot	Inability to walk on left leg, June 2nd
	44	C.N.	5 months	Jan., on right big toe	Inability to move right leg, June 2nd
	45	H.R.	3 months	1st week in Mar., on one of toes, left foot	Pain in left ankle, June 1st
	46	S.R.	3 months	Bitten same night as H.R., on one of left toes	Pain in left ankle, calf and hip, May 31st
	47	J.W.	2 months	Apl. 7th, on left big toe	Numbness in and inability to walk on left foot, June 5th

5. NATURAL INFECTION IN BATS

The capture and destruction of the blood-'sucking' vampire bat as a protective measure in the control of the disease provided specimens for the determination of the infectivity rate as estimated by the presence of Negri bodies. Bats were caught in various parts of the island, irrespective of their species or abnormality in their habits, and the hippocampus was dissected out. The freshly caught ones were examined by smear preparation stained by Muromzew borax methylene blue or Gerlach's method, or fixed in acetone, sectioned and stained by Lentz's ; whereas those preserved in alcohol to avoid decomposition were fixed in Zenker and stained by Mann's method. The results of examination for the presence of Negri bodies are as follows :—

Total no. of bats examined from October 12th, 1934, to September 13th, 1935 .. .. .. = 2,059
No. positive .. .. .. = 64
No. negative .. .. .. = 1,995
Species positive .. .. .. = 59 *Desmodus rot. mur.*, 4 *Artibeus planirostris trinitatis*, 1 *Hemiderma*

Previously examined

No. examined	No. positive
233	24

Total examined = 2,292
Percentage positive = 3·8 per cent.

6. Results of Protection against Bats

As protective measures against the spread of the disease in animals, extensive anti-rabies vaccination, together with ' bat-proofing ' of stables in certain districts, was employed with gratifying results. ' Bat-proofing ' consists in enclosing stables with wire netting to prevent the entrance of bats. The efficacy of ' bat-proofing ' of enclosures is further evidence of the transmission of the disease by bats, as is illustrated in the following specially conducted experiments. In the year 1931, the disease was prevalent in the Maracas district. Mr. ' A.' built two stables 30 ft. apart. One stable was not ' bat-proof ' and contained 4 cattle. Three died from the disease. The other stable was ' bat-proof ' and contained 3 mules and 2 horses. No animal died. Again, at Cedros district, where the disease was also occurring, Mr. ' A.' placed 1 mule and 1 horse in a ' bat-proof ' stable, and allowed 3 donkeys and 1 cow to be loose in the open pasture at night. The former remained well, while the latter all died from the disease.

Again, Mr. ' X.' had ' bat-proofed ' his stables completely and carefully and had kept all his animals indoors at night, yet he suffered losses from the disease. A careful watch was maintained, and it was discovered that vampire bats were crawling beneath a door and entering the ' bat-proof ' stables to bite the animals.

MODE OF INFECTION

Koch (1930) drew attention to the observation of Heller (1904) that dogs are particularly suitable animals for the spread of rabies because their teeth constitute their main weapon of defence. Similarly, the vampire bat is an ideal agent for disseminating infection, inasmuch as to obtain any feed it must inflict a wound with its teeth on man or beast, and, in addition, by its adaptation

for flight it easily overcomes natural barriers to the spread of infection. While a rabid dog departs from its normal habit to bite its master, a vampire will bite whether infected with rabies or not, and the chances of infection are consequently greater. Its teeth are specially modified for cutting or shaving through the skin, the upper incisors, two in number, being extremely sharp, with chisel-like projecting edges, and placed in advance of the canines. The lower incisors are separated to allow the tongue to lap the blood escaping from the wound (Pl. IV, fig. 4). Their mode of attack of human beings has been graphically described by Beebe (1925), and Ditmars and Greenhall (1935) have given a lucid and detailed account of their onslaught on animals. The inhabitants of rural districts have provided the largest number of victims, and the incidence of infection has been greatest upon those of the poorest classes living in unprotected homes (Pl. IV, fig. 2). The wound inflicted on human beings may be oval or circular, superficial or deep. It is usually clean-cut and crater-like, about 2–3½ mm. in diameter, and may penetrate to the muscular tissue. Bleeding may be profuse, due, probably, to an anticoagulant, which was found present in both the dry and fresh powdered salivary gland substance, and which is capable of hindering coagulation of human blood in a greater dilution than 1 in 1,000. The infection is, of course, conveyed in the saliva ; and the salivary glands of the vampire bats of Trinidad have been found infected with the virus of rabies (cf. experiment 9, bat 21). In the histories of bat-bites given by those who have contracted the disease the information is usual that the person was awakened during sleep by feeling a sharp sting at the site of the bite. Examination of the spot would reveal a bleeding wound, and the bat would sometimes be seen flying from around the area bitten. On the other hand, histories of bat-bites previous to the appearance of the disease in the island showed that the person had merely a vague consciousness during sleep that he was bitten by a bat, but no pain was felt. On awakening in the morning, blood or blood-stains would be found on some part of his body. The records of Dr. Rankine, Medical Inspector of Health of the Colony, show that, of 87 persons bitten in the year 1935 by bats, 27 were actually awakened from their sleep by the attack of the bat, whilst the remaining 60 were not aware of the bites until the morning. Ditmars and Greenhall (1935) quote Dunn as saying that in Panama the younger bats are not so expert in effecting their bites, and thus cause pain. But may this difference in the fierceness of attack between a healthy and a rabid bat be due to infection with the virus ?

The abnormal flights of bats which so impressed the inhabitants of South Brazil in 1914–18 have also been seen in Trinidad, and detailed accounts have been obtained on 18 occasions from reliable witnesses. These accounts describe bats flying about in a frenzied manner during the day, and dashing themselves against walls and other barriers, contradicting Spallanzani's experiments, which demonstrated the highly delicate sense of touch possessed by Chiroptera. In 1932 ' a bat was seen attacking a woman in an open yard and persisting in flying

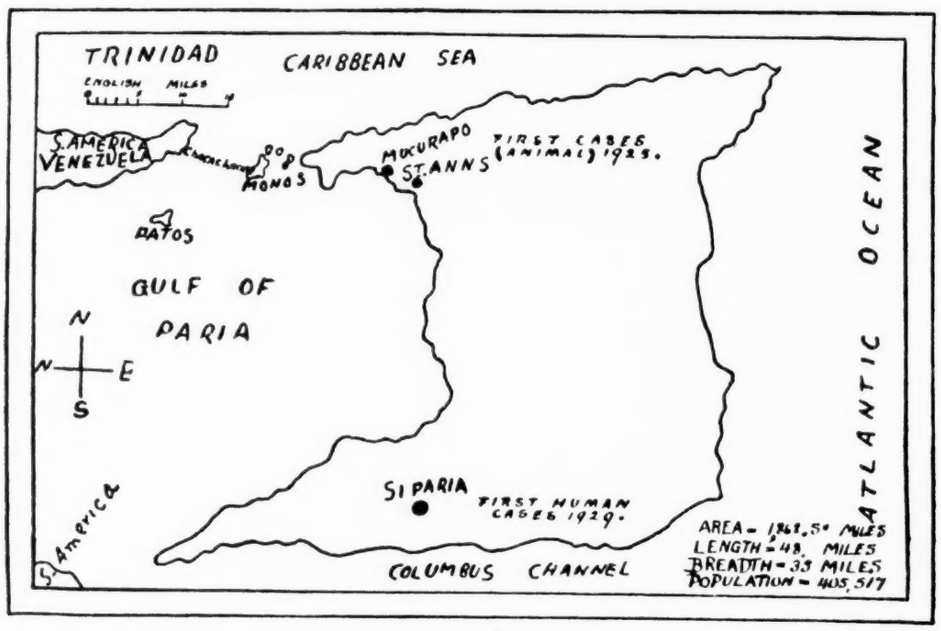
at her in spite of her efforts to frighten it away. In response to her screams a man came to her rescue with a broom, but the bat then attempted to attack both of them. Eventually it was knocked down with the broom.' Animal inoculation showed that this bat was infected. Again, in the same year a bat was seen repeatedly to attack a goat in the day ; the goat warded off the attempts to bite by butting at the bat, but the latter persisted in its efforts and was stoned and killed by some onlookers. On the other hand, there are instances where bats would merely bite or snap ' like a dog ' at an animal, without lapping any blood. In such cases only a small punctured wound, not easily detectable, would be left. It has been noted in Trinidad that reports of such abnormal aggressiveness is associated with periods of prevalence of paralytic rabies in man or animals, and the opinion of Haupt and Rehaag that these ' biting fits ' must be regarded as a sign of disease seems therefore justifiable.

DISCUSSION

The island of Trinidad lies about 12 miles distant in the north, and 9 miles in the south, from the mainland of South America, where rabies is known to exist. A series of islets in the north link it to the mainland, while in the south there is an unbroken expanse of sea. Its quarantine laws controlling rabies demand 6 months' isolation of all canines before admission to the island, except in the instance of animals introduced from Great Britain. The last reported case of canine hydrophobia in man occurred in 1912. In 1911 there were four cases. There has been no other since. The first known cases of paralytic rabies in this outbreak occurred in Mucurapo and St. Ann's, in the north part of the island about a quarter of a mile to 2 miles from the sea-coast facing the South American continent. The sudden appearance of rabies in Trinidad in 1925 may therefore be explained on one of two assumptions : (1) the flight, or transit in sloops and other vessels, of infected bats from the mainland ; (2) the infection of bats in Trinidad by some latent, unrecognized ' carrier ' of the disease.

The epidemic of paralytic rabies which swept over Brazil made its first appearance suddenly on the sea-coast of Santa Catharina, South Brazil, and travelled northwards over the continent to the borderland of Venezuela and British Guiana. Haupt and Rehaag (1921) suggested that an infected canine in Biguassu, South Brazil, had bitten a bat, which in turn had spread infection among other bats. Paralytic rabies does exist in animals in the hinterland of Venezuela and the Guianas, but, as far as is known, no case has appeared on the coast opposite to Trinidad. Bats have, however, been seen by a reliable authority winging their flight across from the mainland towards Trinidad, and *Desmodus* exists in the island of Chacachacare, situated about midway between Venezuela and Trinidad. On the assumption of the passage of an infected bat from South America, the appearance of the first cases in 1925 in Mucurapo

Pasture and St. Ann's will be explicable by reference to Map 1. But it is also possible that infection of bats arose from some latent, unrecognized source in the island itself. The records of the Colony show that attempts in the distant past to establish ' hatos ' or stock-farms in the neighbourhood of the villages of Erin and Siparia failed, owing, it has been stated, ' to the ravages of bats.' In the year 1889, a distinguished chemist (J. M. C.), while engaged locally in dissecting vampire bats to determine the nature of the stomach contents, developed a fatal paralysis which was certified as ' acute ascending spinal paralysis.' In 1919—in the continued absence of recognized rabies—a peasant girl and a woman in the central part of the island were bitten in the lower extremities by a ' strange, mad ' cat. Six weeks later, they both developed ' a severe



MAP 1

burning and pain at the site of the bite,' followed by an acute spreading paralysis, great thirst, and inability to swallow, with death in 3 days. Hydrophobia was diagnosed by the Medical Officer, but no further investigation was undertaken. The vampire bats of Trinidad have been biting human beings and animals for the last 60 years at least, and, whatever may have been the original source of their infection with rabies, the probability is that this infection took place in the neighbourhood of Mucurapo and St. Ann's in the north of the island, some time in the year 1924 to 1925.

The Trinidad outbreak, in contrast to the Brazilian epidemic, has involved both human beings and certain animals. The infected *Desmodus* exists in South America, bites and infects animals, and also bites human beings ; but so far no case has been reported in man. The Brazilian bat strain therefore differs from the Trinidad bat virus in its innocuousness to man. Such a selective

infectiousness is not peculiar to South America, for Bouffard (1912) in Senegal, Remlinger and Curasson (1924) in French North Africa, and Delpy, Cauvin and Riou (1929) in the French Sudan, and others, have shown that, though canine rabies does exist in French Equatorial Africa, the disease does not affect



Map 2

human beings. Nicolau, Mathis and Constantinesco (1933) came to a similar conclusion by demonstrating definitely that 'Oulou-fato' of French West Africa is canine rabies in indigenous dogs, and that this form of rabies is almost invariably of the paralytic type. Further, though it existed side by side with

classical rabies, it is not known to be transmissible to man. Bouvier (1934) also described paralytic rabies in dogs in the Congo. Human beings have been bitten, but no case has terminated fatally. In Trinidad from 1925 to 1929 the outbreak of paralytic rabies was confined solely to animals, no case having been reported in man. But suddenly, for some reason unknown, 13 human beings developed the disease in the village of Siparia, after it had existed for 4 years among animals throughout the Colony. Did some unrecognized factor in Siparia in the south of the island modify the virus in the bat, rendering it pathogenic to man ? Unfortunately, no bat strain recovered previous to the appearance of the disease in human beings is available for comparison with the present human-bat virus, though a study of the Brazilian bat strain with the Trinidad one would be of interest.

Haupt and Rehaag (1921) consider ' fighting and shrieking ' of bats to be evidence of disease. In Trinidad such tendencies have been found associated with infection with rabies, but flying during the day is not necessarily a symptom of disease. Bats have been seen flying during the day by the earliest observers in various parts of the tropics, and such abnormal flight was not associated with an undue mortality among them, nor with morbidity in man or beast. Bats caught flying during the day may not only reveal no Negri bodies, but may fail to produce rabies in experimental animals. Lima (1934) showed that a *Desmodus*, infected experimentally with bovine virus, can remain infective without any evidence of disease for 1 to 5 months. In Trinidad, bat 4290 was kept in captivity for 161 days, i.e., nearly $5\frac{1}{2}$ months, and exhibited no abnormal symptoms ; yet it had been infected in nature and remained infective under artificial conditions during that period. It has, however, been observed in Trinidad that an unusual prevalence of biting of animals or human beings in a district usually precedes the development of the disease, and this is now, therefore, regarded as an abnormal manifestation, suggesting infection of bats of the district with rabies—in addition, of course, to the evidence of undue aggressiveness and fighting propensities.

The importance of Negri bodies in the diagnosis of rabies was obscured temporarily by the announcement of Kraus (1932*a*) that material showing Negri bodies and originating from supposed cases of rabies in Santa Catharina, sent by Haupt and Rehaag through Carini, was found by Negrette, Kantor and himself in 1918 to be infected with the virus of infectious bulbar paralysis (Aujesky's disease), and not with rabies. Remlinger (1931*a*) utilized this finding to emphasize the pitfalls in the differentiation, without recourse to cross-immunity and serum neutralization tests, of various neurotropic viruses allied to rabies. Subsequently, however, Kraus and Duran and Ortiz Patto (1932) stated that both pseudo-rabies and true rabies were occurring simultaneously in Santa Catharina, and that the material studied by Negrette and Kantor was recovered from a case of Aujesky's disease. Specimens of brains from bats in which no Negri bodies could be found have produced emaciation with paralysis and

death in rabbits in 5 to 12 days after intracerebral inoculation, but the most careful examination could reveal no Negri bodies in these rabbits. Whether the results obtained in Trinidad are due to rabies or infection with some allied condition is at present being investigated. Carini (1911) himself reports the failure to find Negri bodies in the first rabbit passage of material which had shown numerous bodies ; and in Trinidad, while all human cases were positive in this respect (except the two doubtful ones in 1933) Negri bodies are at times absent in both bovines and equines showing the typical paralytic symptoms of rabies contracted in the field. Pirani (1929) has given an account of rabies in Eritrea in man, donkeys, dogs and cats, etc., in which Negri bodies were, in most instances, absent. So also in Roumania, Jonnesco (1932) encountered a strain of street virus of exalted virulence, causing rabies in rabbits with a constant incubation period of 3 days, followed by death in from 1 to 3 days ; but Negri bodies could not be found in any of the rabbits. The Koritschoner virus, as well as the virus ' D K,' as stated by Kraus, Gerlach and Schweinburg (1926), and by Remlinger and Bailly (1931*a*), also showed no Negri bodies; so also the strain of canine virus in the Congo described by Bouvier (1934).

Of the bats so far found in Trinidad with Negri bodies, 5 were *Artibeus*, 1 *Hemiderma* and 1 *Diclidurus*. These are not blood-lappers, but fruit- and insect-eaters. Communities of bats may consist of more than one species, and frugivorous and insectivorous bats may be captured in the same habitat as vampires. The spread of infection among them by the *Desmodus* is thus explicable. In Brazil, as in Trinidad, the first bat found with Negri bodies was not a vampire. Haupt and Rehaag, in Blumenau, showed that a *Phyllostoma superciliatum* Burmeister was infected with rabies, in addition to the *Desmodus* ; and in Trinidad an *Artibeus*, a fruit-eater, was the first bat discovered with Negri bodies. Phyllostomidae or Vampiridae are the so-called false ' vampires.' They are fruit-eaters and—contrary to the belief in certain quarters—not blood-' suckers.' Infected Desmodontidae by their blood-' sucking ' propensities transmit rabies readily, whereas only through a change in their diet and mode of life can the frugivorous and insectivorous species infect man or animals. But does infection of a ' non-blood-sucker ' with rabies lead to an alteration in its habit and the development of biting propensities ? No evidence is as yet available to incriminate non-blood-suckers as direct active agents in the spread of the disease in Trinidad.

The remarkable immunity enjoyed by dogs in Brazil in the presence of a widespread outbreak of rabies in herbivorous animals was emphasized by Carini, Haupt and Rehaag, Kraus and Duran, and others. In Trinidad this freedom of canines from infection is also of special significance, for the number of stray, ownerless dogs sleeping in the open—and many in closest proximity to affected animals known to be bitten by bats—suggests that the local virus is innocuous to canines, or that dogs are never bitten in their sleep. McKendrick (1932) drew attention to the fact that certain species of animals (e.g., the rat),

though freely susceptible to rabies under laboratory conditions, very rarely transmit the disease in nature. This fortunate circumstance may be due to the habit of the animal, and not to the absence of infected ganglion cells as suggested by Manouélian and Viala (1927). Du Toit (1929) described the sudden appearance of rabies in South Africa in 1913, and its spread by the mongoose and meerkat—both wild carnivores. Human beings and domestic animals have been affected, but, in spite of exposure to infection, dogs have not contracted the disease. Kraus, Gerlach and Schweinburg (1926) have also pointed out the comparative rarity of canine rabies in Turkey. In the experiments here recorded it is seen that bat 10 was injected into 2 dogs on the same day and under similar conditions. There was no regurgitation of material, but one animal developed paralytic rabies, while the other did not. Strains of the virus isolated from both human beings and animals readily produced paralytic rabies in dogs in Trinidad, whereas similar material from human beings induced furious rabies at the Rockefeller Institute, New York (1932), and Remlinger and Bailly (1932), working in Morocco with a bovine strain of the Trinidad virus, produced both furious and paralytic rabies in dogs. Is there some immunity on the part of dogs in Trinidad and Brazil modifying or rendering the bat virus harmless ?

It is also of interest to refer to the constancy of the development of paralytic symptoms without ' furious ' manifestations in all the experimental animals—except in one instance with bat S.I. Under natural conditions the disease in both human beings and animals in Trinidad was also of the paralytic form—possibly not in 2 cases in bovines. Haupt and Rehaag suggest that the virus undergoes some modification in the bat, rendering it liable to produce only ' dumb ' rabies. Another explanation may be possible. Street virus from a case of ' furious ' rabies after repeated intracerebral passages in a mammal becomes a ' *virus fixe*,' one of the characteristics of which is the production of paralytic, and not furious, symptoms. Perhaps the unbroken passage through several generations of bats produced a ' *virus fixe* ' for bats of exalted virulence which infects only in a paralytic form. This theory receives some support from the close similarity in the symptoms of the disease in bovines in the laboratory and in nature. From January, 1932, to September, 1935, 472 calves have been inoculated intracerebrally with ' *virus fixe* ' received from the Ministry of Health, England, for the preparation of anti-rabies vaccine. In every instance the symptoms were identical with those seen in bovines infected by bats under natural conditions, and also with those infected subcutaneously and intracerebrally with bat virus in the laboratory. But if the virus has been fixed in the bat, Negri bodies should not be detected by Mann's method, despite the work of Nicolau and Kopciowska (1932) on the presence of Negri bodies in animals infected with ' *virus fixe*.'*

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*This was written before the appearance of the article by S. Nicolau and L. Kopciowska entitled ' Sur la Rage ' (*Ann. Inst. Pasteur*, 1935, *Supplément*).

While the experimental evidence here submitted demonstrates that bats, and especially the *Desmodus*, are infected with the virus of rabies, the definite and conclusive testimony contained in the clinical histories of the human cases prove that the *Desmodus* bats are, in truth and in fact, the agencies by which the disease is being spread. The regular history of a bat-bite previous to the development of symptoms, and the localization of the first symptoms to the site of the bite, are conclusive in their significance. In this respect case P., no. 23, is of interest. She was bitten on the right elbow in the same room and on the same night as her child, who was bitten on the foot. Her first symptoms appeared in the elbow and his in the foot. Case no. 31, ' C. B.', does not permit an explanation, for, though he was bitten on the right foot, the earliest manifestation developed in the right *upper* extremity. Cases of paralytic rabies in which the paralysis developed in a limb other than the one bitten are not unknown. Knutti (1929) quotes Gamaleia as reporting such cases, and McKendrick (1935) quotes Dorolle, Chaussinand and Tam (1935) as having recently studied a somewhat similar case at Saigon. The patient was bitten on the thumb by a dog with furious rabies. He developed pain at the site bitten, followed later by an ascending paralysis of the *lower* extremity with death. Case no. 39, ' T. O.', is unusual, for, despite the most careful interrogation, he denied ever having been bitten by a bat—except about 20 years ago, when, he said, he was bitten on the foot. It is noteworthy that his hippocampus, cerebellum and optic nucleus swarmed with Negri bodies. Neither in animals nor in any other human being were they so numerous. Instances of human beings suffering from classical hydrophobia and being unmindful of ever having been bitten by a dog or other animal are not unknown, as in the remarkable case reported by Knutti (1929). But this isolated instance does not negative the body of histological, experimental, clinical and epidemiological evidence incriminating the vampire bat as the transmitting agent of paralytic rabies in Trinidad. While everybody who developed the disease (except case 39) was bitten by a bat, no person bitten by a bat contracted the disease after a course of anti-rabies vaccination. The statement, accepted unquestionably, that all mammals are susceptible to rabies, made without reference to bats—those strange mammals—can now therefore be received unreservedly.

SUMMARY

1. In the year 1925 paralytic rabies appeared among certain animals in the island of Trinidad, and in 1929 affected human beings.

2. Human beings bitten by vampire bats develop sensory symptoms at the site bitten, followed by paralysis and death.

3. Vampire bats by histological examination, animal inoculation, cross-immunity, and serum neutralization tests have been found infected with the virus of rabies.

4. The conclusion is drawn that the vampire bat (*Desmodus rotundus murinus* Wagner, 1840) is the transmitting agent of the disease in man and animals.

To Dr. K. S. Wise, Surgeon-General of the Colony, grateful acknowledgment is made for his many helpful suggestions and kindly criticisms, and for the abundant facilities so readily placed at my disposal. To Captain Metivier and the other veterinary surgeons who have so willingly supplied material for study, I wish to express my indebtedness. To those Medical Officers, especially Dr. R. C. Wupperman, Dr. G. C. Deane and Dr. P. A. Rostant, whose co-operation has been of great value, and to Mr. F. W. Urich, who kept one of the bats in captivity for 5½ months, my thanks are also due.

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EXPLANATION OF PLATE IV

Fig. 1. Abandoned sugar-mill, harbouring infected *Desmodus* bats, Arranguez Estate, San Juan.

Fig. 2. ' Barracks ' house, Santa Cruz, in which case no. 41, ' N. S.', was bitten.

Fig. 3. A non-blood-' lapper ' bat, fruit-eater, *Artibeus planirostris trinitatis*.

Fig. 4. A blood-' lapper ' vampire bat, *Desmodus rotundus murinus* Wagner, 1840.



FIG. 1

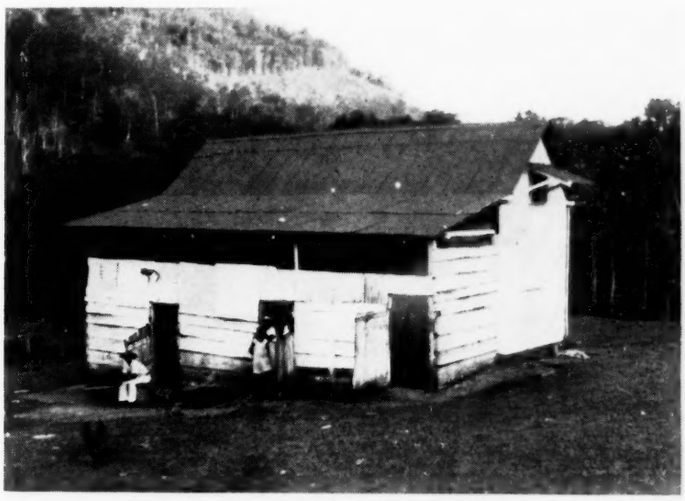


FIG. 2



FIG. 3



FIG. 4

H. R. Grubb, Poplar Walk, Croydon

MISCELLANEA

PRELIMINARY RECORDS OF LARVAE OF THE *FUNESTUS* SERIES COLLECTED IN KENYA BY MR. C. B. SYMES

Recently Mr. C. B. Symes has undertaken jointly with the writer a survey of the larvae of the *funestus* series in Kenya.

Larvae resembling those of *A. leesoni* and the two recently described varieties of *funestus* had been recognized among material sent by Mr. Symes, and the first sixteen collections of larvae recently received from eleven different localities indicate that two of these forms are evidently fairly well represented in the Colony, though they are probably not nearly as numerous as in Southern Rhodesia.

Anopheles funestus type form. This is evidently the greatly predominating form of the series in the Colony. In five collections it was the only larva present, and in two others it was much the most numerous. In some of the collections the tergal plates showed rather marked deviation from the usual condition.

In the larvae from one locality the tergal plates mostly showed a darkened central area contrasting with paler lateral portions. Among the larvae from another district an appreciable proportion of specimens had the paired accessory plates projecting to some extent from the posterior margin of the main plate on several of the segments, sometimes including the fifth and sixth.

Anopheles funestus var. *rivulorum* Leeson. Larvae agreeing closely with those of this form from Southern Rhodesia occurred in three localities. The only important difference noted was the complete absence in most of the specimens of the paired accessory tergal plates.

Anopheles funestus var. *confusus* Evans and Leeson. A single larva agreeing with that of this form was identified ; locality unspecified.

Anopheles leesoni Evans. The larvae of this series received from three localities agreed exactly with South African material of this species.

10 *February*, 1936. A. M. Evans.

CONGO SIMULIIDAE

BY

E. G. GIBBINS

(*Received for publication* 2 *March*, 1936)

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INTRODUCTION

Our meagre knowledge of the Simuliid fauna of the Belgian Congo has been greatly enhanced by the recent researches of Dr. J. Bequaert, who, with the assistance of Dr. J. Hissette, reared a large number of Simuliid flies, while engaged in a study of their relationship to onchocerciasis during the sojourn of the Harvard Expedition in 1934. Dr. Bequaert very kindly placed his material, together with a small collection which he had obtained from the Congo Museum at Tervueren in Belgium, at the writer's disposal. Its subsequent examination showed the presence of some 17 different species, two of which proved new to science and revealed the individuality of *Simulium violaceum*, hitherto regarded as a variety. Furthermore, the collection contained examples of *Simulium nigritarsis* and *Simulium alcocki*, which make it possible to supplement previous descriptions of these two species.

While the pupa has been fully dealt with in the case of each species, the description of the external characters of the adult should be taken with caution, as owing to the specimens being preserved in spirit much of the pubescence was lost. None of the larvae are known.

Simulium bequaerti sp. nov.

FEMALE. Isolated with its pupal pelt and cocoon.

Head. Vertex and *front* grey with yellow scales ; front of normal width. *Antenna* as in male.

Thorax. Mesonotum brown with golden scales. *Pleura* and *halteres* as in male.

Abdomen brown with sparse yellow scales ; first tergite as in male but with shorter hairs. *Eighth sternite* without a median chitinized area.

Terminalia (fig. 1). *Anterior gonopophyse* (fig. 1, *b*) rounded and lightly chitinized ; paraproct (fig. 1, *a*, *c*) small and tapering to a rounded end medially. *Cercus* large and flattened distally.

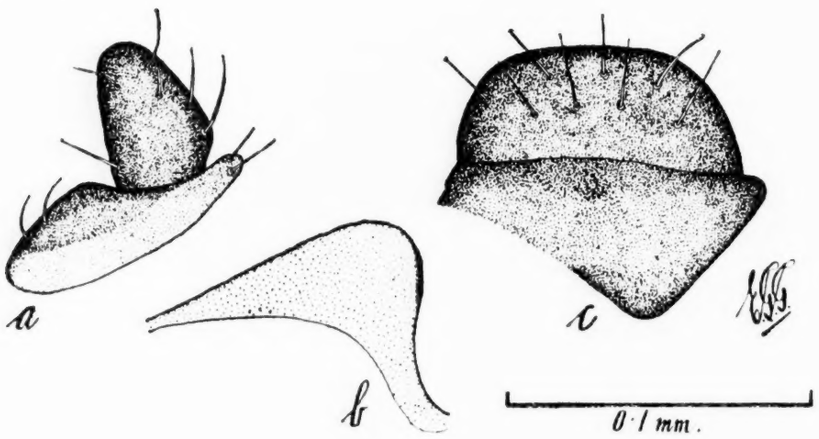


FIG. 1. *S. bequaerti.* Female terminalia. *a.*—Ventral view of paraproct and cercus ; *c.*—Lateral view ; *b.*—Anterior gonopophyse.

Wing with a basal cell, hairs on the subcosta and base of radius.

Legs as in male, except that the basal two-thirds of the hind first tarsal segment is pale.

MALE. Isolated with its pupal pelt and cocoon. Length in spirit 2·5 mm. ; wing 2 mm.

Head. Eyes closely approximated. *Antenna* brown, with the exception of scape, first and base of second segment, which are light brown.

Thorax. Mesonotum velvet-black with scanty golden scales. *Pleura* without patch of hairs on the membrane behind the thoracic spiracle. *Halteres* pale yellow.

Abdomen velvet-black with scanty golden scales ; first tergite brown with fringe of long dark-brown hairs. *Terminalia** (fig. 2). *Coxite* (fig. 2, *a*) large and broad in ventral aspect, slightly wider anteriorly than proximally and rather longer on its inner side. *Clasper* small and rounded distally, with a small chitinized tooth on its lower apical edge. *Phallosome* : *anterior part* (fig. 2, *b*) broad in ventral view and rounded distally, with short setae and with a lightly chitinized area produced basally to a point ; in lateral view (fig. 2, *c*) it is narrow with a slight posterior bulge ; *posterior part* (fig. 2, *d*) consisting of a pair of large, strongly chitinized, spine-like parameres with a short broad apodeme. *Cercus* (fig. 2, *e*) long, with about 7 setae arranged along the edge.

Legs brown, with the exception of the basal two-thirds of the femora and base of the tibiae of the hind legs, which are pale. Claws with a basal tooth.

---

* The male terminalia of *Simulium* was dealt with in a recent study (1935).

TYPE male, on slide. Kundelungu Mts. (Katanga), west of Lake Moero, source of River Mushipushi. October, 1934. Dr. J. Hissette.

ALLOTYPE female, on slide. Luputa (Kasai). Dr. J. Hissette.

LARVA unknown.

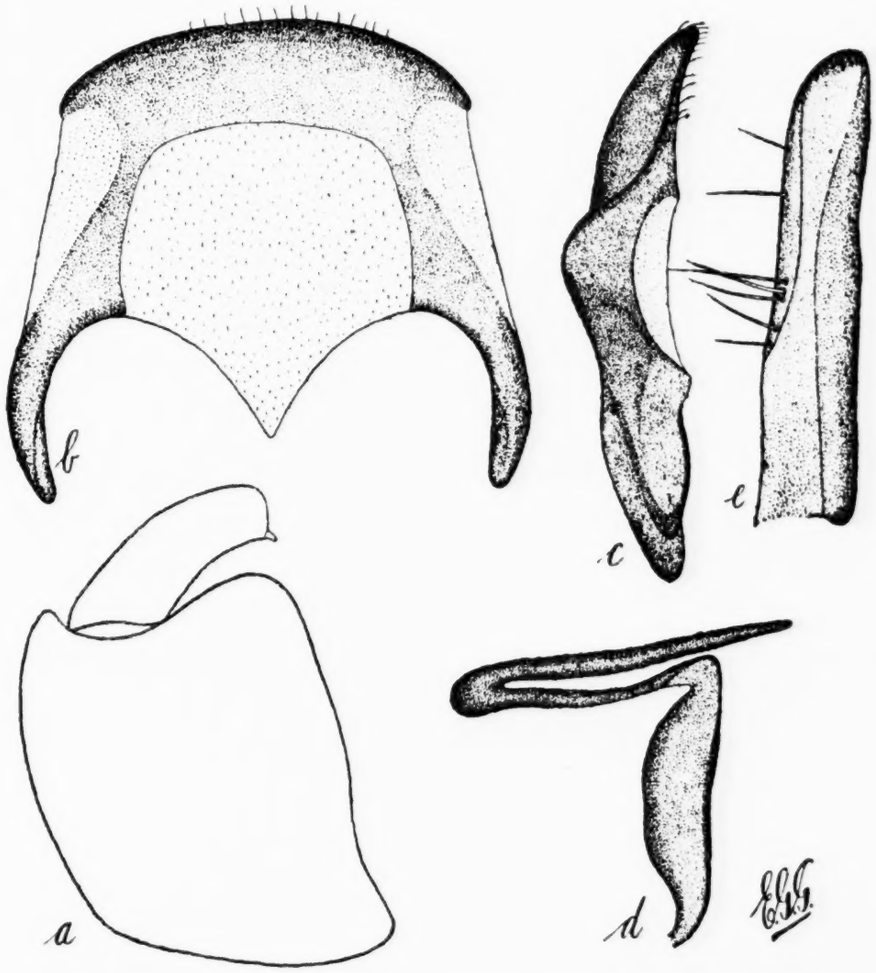


FIG. 2. *S. bequaerti*. Male terminalia. *a*.—Ventral view of coxite and clasper ; *b*.—Phallosome, anterior part, ventral view ; *c*.—Lateral view ; *d*.—Posterior part, dorsal view of right side ; *e*.—Cercus. (Magnification of *a* as in fig. 1 ; of *b*, *c*, *d*, *e* as in fig. 10.)

PUPA.

Head and *thorax* with disc-like tubercles and short simple trichomes.

Respiratory organ (fig. 3, *a*) with a long main stout stem bearing a deeply chitinized irregular worm-like pattern (fig. 3, *b*), and with eight slender semi-translucent filaments which arise from two short stems ; one gives rise to a single pair of long filaments, and the other to two secondary stems, an inner which is situated medially and divided dichotomously into two pairs, and an outer which gives rise to a single pair of filaments.

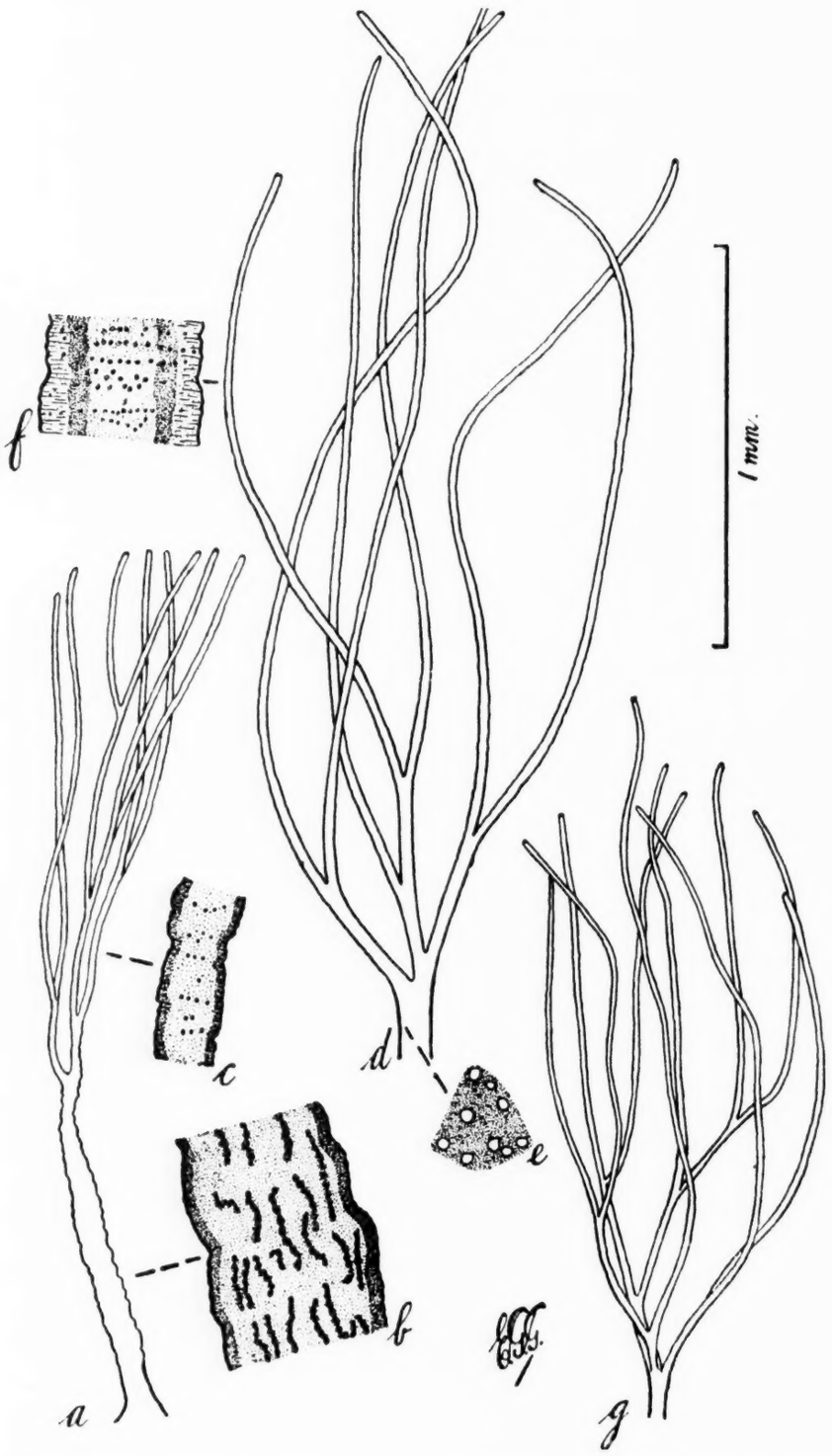


FIG. 3. Respiratory organ of pupa. *a*.—*S. bequaerti* ; *b*.—Sculpture of main stem ; *c*.—Filament ; *d*.—*S. alcocki* ; *e*.—Sculpture of base ; *f*.—Filament ; *g*.—*S. violaceum* ; sculpture of base and filament as in *S. alcocki*. (Magnification of *c*, *f*, *e* as in fig. 10 ; of *b* as in fig. 7, *c*.)

Abdomen. Terminal segment with a strong pair of downward-projecting spines. Dorso-lateral surface : third and fourth segments with 4 closely placed strong hooks ; sixth with 3 ; and seventh and eighth with 4 short downward-projecting spines ; ninth with a basal row of short spines. Ventro-lateral surface : fourth segment with a closely placed simple spine and a bifid hook ; fifth with a pair of closely placed bifid hooks ; sixth and seventh with a pair of widely placed bifid hooks.

Cocoon (fig. 4, *b*, *c*). 1·5 mm. in length, gelatinous and interwoven with coarse threads.

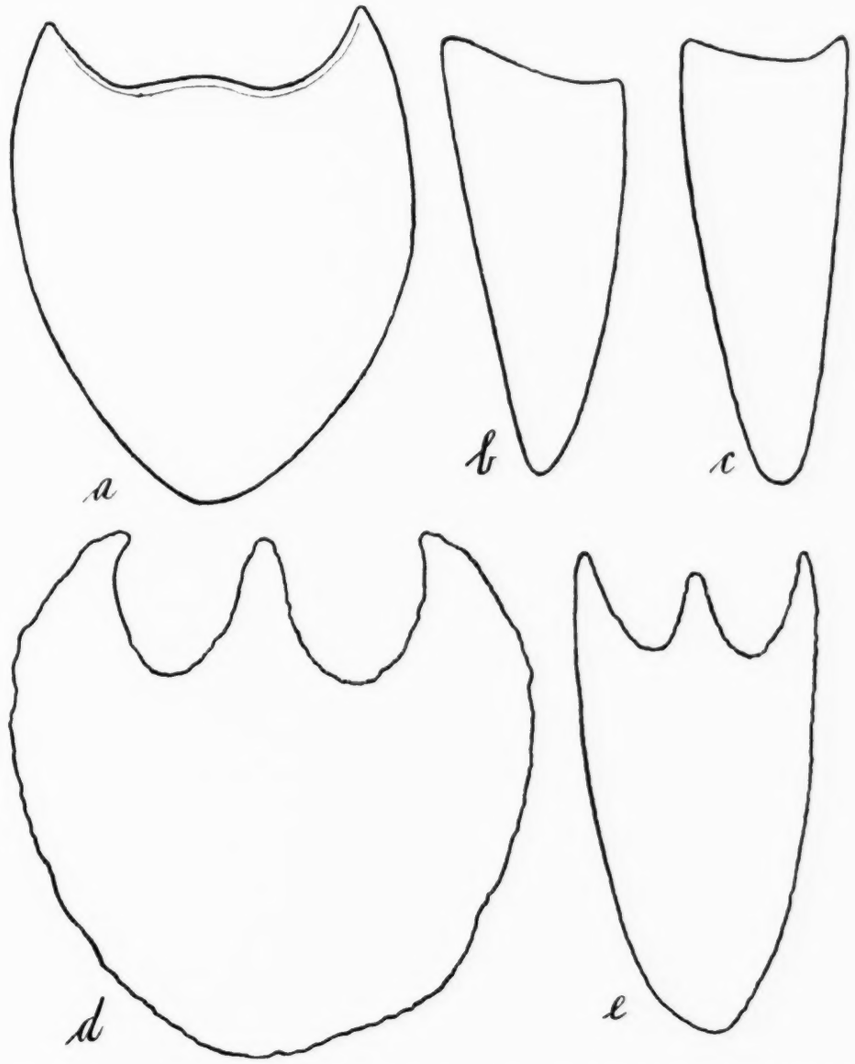


Fig. 4. Cocoon. *a.—S. nigritarsis*, ventral view ; *b.—S. bequaerti*, lateral view ; *c.*—Ventral view ; *d.—S. hissetteum*, ventral view ; *e.—S. alcocki*, ventral view ; cocoon of *S. violaceum* as in *alcocki*. (Not drawn to scale.)

The species is easily distinguished from all other Ethiopian Simuliids on the character of the male terminalia and the respiratory organ of the pupa.

Simulium hissetteum sp. nov.

Closely related to *S. bequaerti* and exhibiting no apparent external differences in the male and female in specimens preserved in spirit, but differing markedly in the coxite and clasper of the male terminalia and in the respiratory organ of the pupa.

FEMALE. Length in spirit 2 mm. ; wing 2.2 mm.

Abdomen. Spermatheca and *terminalia* as in *nigritarsis.*

Wing with a basal cell, hairs on the base of the radius and along almost the whole length of the subcosta.

MALE. Dissected from mature pupa.

Abdomen. Terminalia (fig. 5). *Coxite* (fig. 5, *a*) large, about as long as the clasper and somewhat triangular in shape in ventral view ; clasper tapering to a rounded end, with a chitinized tooth at the tip. *Phallosome* : *anterior part*, ventral aspect as in *alcocki* ; lateral view (fig. 5, *b*) broad and rounded posteriorly with a short downward-projecting process; median process as in *alcocki* but slightly longer ; *posterior part* and cercus as in *alcocki.*

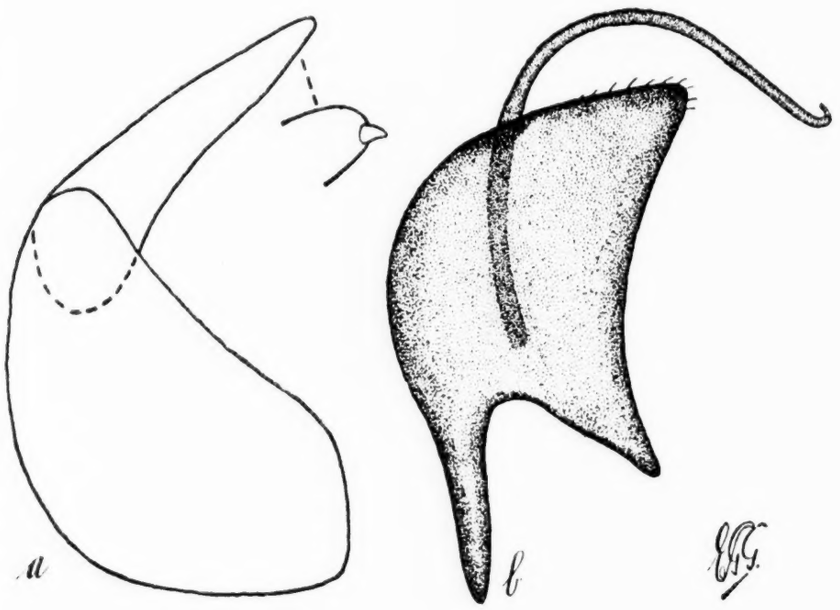


FIG. 5. *S. hissetteum.* Male terminalia. *a.*—Coxite and clasper, ventral view ; *b.*—Phallosome, lateral view of anterior part ; ventral view of phallosome and cercus as in *S. bequaerti* (fig. 2). Note tip of clasper drawn from a lateral aspect. (Magnification of *a* as in fig. 1 ; of *b* as in fig. 10.)

TYPE male, on slide. Luputa (Kasai). Dr. J. Hissette.

ALLOTYPE female, on slide, same data.

LARVA unknown.

PUPA.

Head and *thorax* with disc-like tubercles and simple trichomes.

Respiratory organ (fig. 6) about as long as the cocoon, consisting of a tubular structure resembling an inverted test-tube, with an outer wall having the reticulated appearance shown in figure 6, *b*, and 16 long, slender, semi-translucent filaments of uniform length, which arise at irregular intervals in the anterior two-thirds, principally from the ventral surface.

Abdomen. Terminal segment with a pair of strong hooks. Dorso-lateral surface : third and fourth segment with 4 strong hooks ; seventh with 4 ; and eighth with 8 strong downward-projecting spines of irregular size. Ventro-lateral surface : sixth and seventh segments with a pair of closely placed bifid hooks ; eighth with a simple bifid hook.

COCOON (fig. 4, *d*). A gelatinous shield-shape network 3 mm. in length, with an irregular outer edge and a median anterior projection.

Simulium nigritarsis Coquillett

The species, which was first described by Coquillett in 1901, was redescribed by De Meillon (1930), who also dealt with the respiratory organ of the pupa and the male terminalia (1934). The male terminalia is dealt with in greater detail below, and the female terminalia and the pupa are fully described from specimens obtained by Dr. Bequaert at Elisabethville. The species is closely related to *S. ruficorne*, *S. brachium* and *S. simplex* (Gibbins, 1936*a*), in which the respiratory organ of the pupa has four stout filaments and the terminalia are of similar type.

FEMALE.

Abdomen. Spermatheca sub-spherical and lightly chitinized, duct unchitinized. *Eighth sternite* with a lightly spiculate median area.

Terminalia (fig. 7). *Anterior gonopophyse* (fig. 7, *a*) rounded and curved, with fine setae. *Paraproct* short and broad (fig. 7, *b*, *c*), showing little reduction in size distally. *Cercus* long and narrow.

MALE.

Abdomen. Terminalia (fig. 8). *Coxite* (fig. 8, *a*) about half as long again as the clasper, which is expanded apically and bears a small pointed chitinized tooth. *Phallosome* : *anterior part* (fig. 8, *b*) of the *ruficorne-brachium-simplex* type, differing from these species in the presence of an anterior flap-like process on either side ; this is best seen when the part is examined from a lateral aspect (fig. 8, *c*) ; median process short and expanded distally ; *posterior part* (fig. 8, *d*) partly membranous, with strongly chitinized spine-like parameres about as long as the apodeme. *Cercus* rounded, with long setae arranged as in fig. 8, *e*.

LAVA unknown.

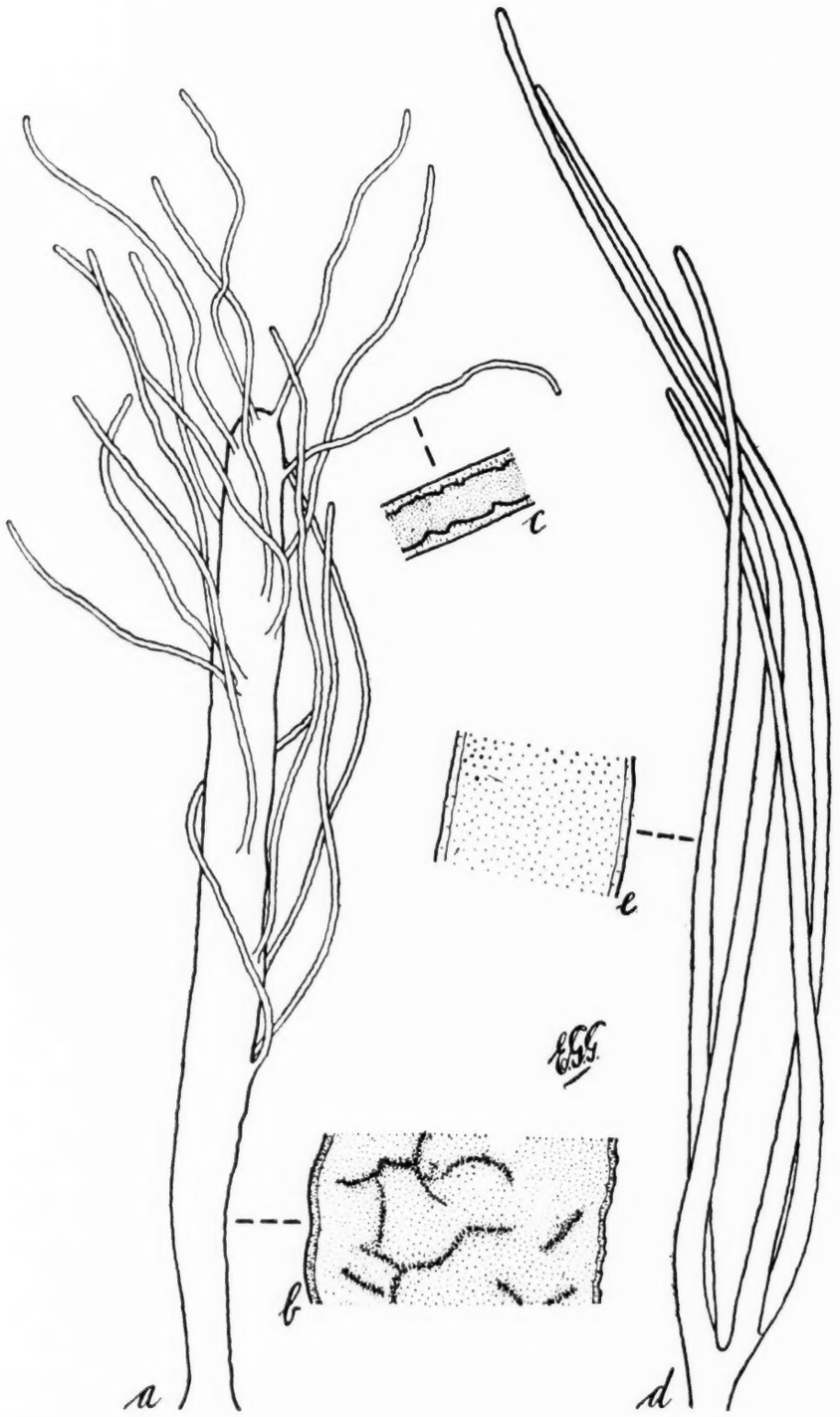


FIG. 6. Respiratory organ of pupa. *a.*—*S. hissetteum* ; *b.*—Sculpture of main stem ; *c.*—Filament ; *d.*—*S. nigritarsis* ; *e.*—Sculpture of filament. (Magnification of *b* as in fig. 7, *c* ; of *c*, *e* as in fig. 10.)

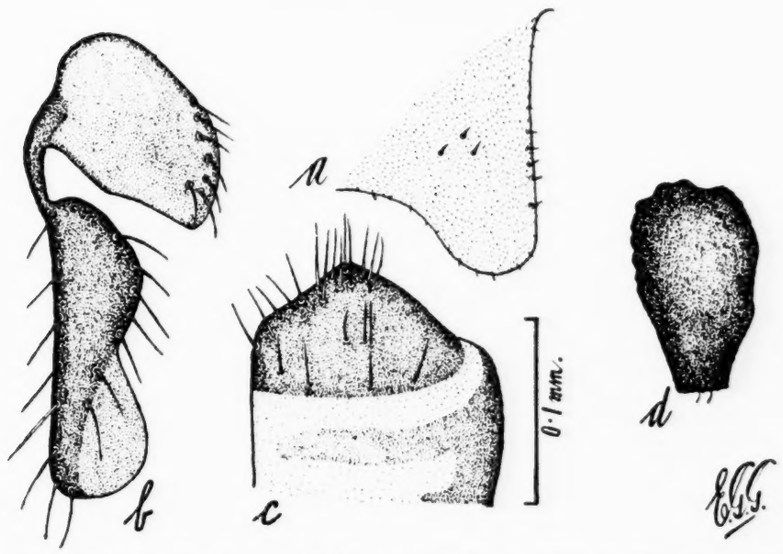


FIG. 7. *S. nigritarsis*. Female terminalia. *b*.—Ventral view of paraproct and cercus ; *c*.—Lateral view ; *a*.—Anterior gonopophyse ; *d*.—Spermatheca of *S. violaceum*. (Magnification equal in *c* and *d* ; of *a*, *b* as in fig. 10.)

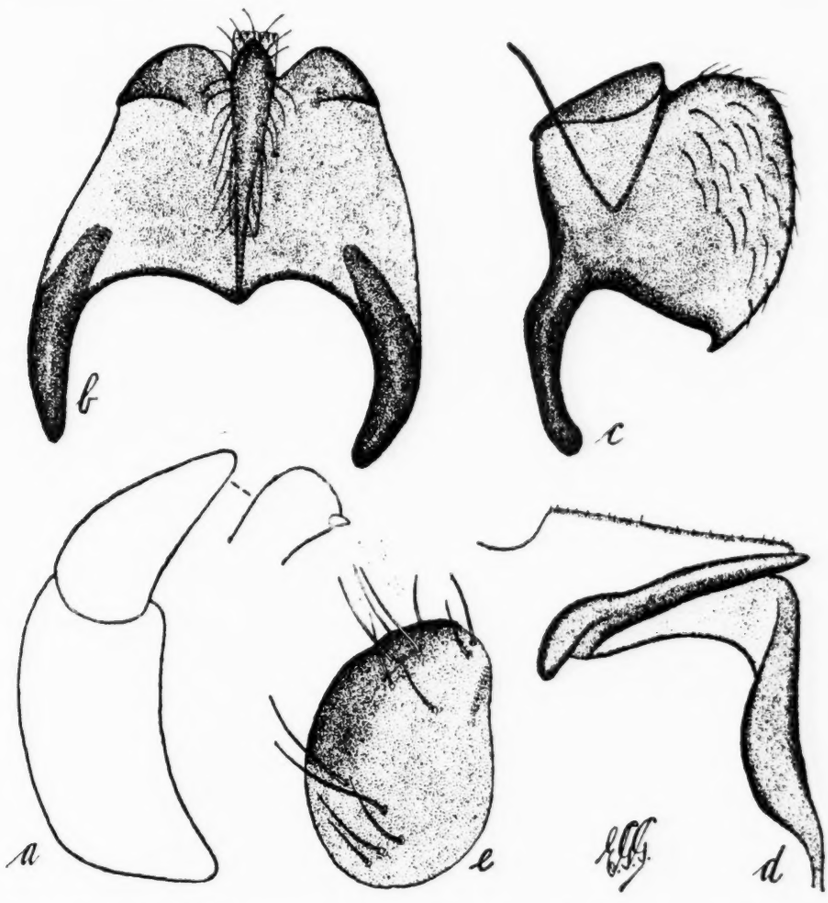


FIG. 8. *S. nigritarsis*. Male terminalia. *a*.—Coxite and clasper, ventral view ; *b*.—Phallosome, anterior part, ventral view ; *c*.—Lateral view ; *d*.—Dorsal view of right side of posterior part ; *e*.—Cercus. Note the tip of the clasper in lateral view. (Magnification of *a* as in fig. 7, *c* ; of *b*, *c* as in fig. 1 ; of *d* as in fig. 10.)

PUPA.

Head and *thorax* with sparse disc-like tubercles and short simple trichomes. *Respiratory organ* (fig. 6, *d*) consisting of four semi-translucent fragile filaments with an outer wall, as depicted in fig. 6, *e*. They are paired, and arise from a short base bearing disc-like tubercles, as in the case of *alcocki* (fig. 3, *e*). The filaments are of unequal length, and in each pair there is a long and a slightly shorter filament, the larger being slightly longer than the cocoon.

Abdomen. Terminal segment with a pair of downward projecting hooks. Dorso-lateral surface : first segment with a row of 4 slender minute spines, the outer slightly separated from the rest ; third and fourth segments with a row of 4 equally spaced strong hooks ; sixth, seventh and eighth with a row of downward-projecting spines, the former with 7, the inner pair of which are distinctly shorter than the rest, and the latter two with a row of 5 and 6 spines respectively ; ninth with a basal row of minute spines. Ventro-lateral surface : second segment with a single strong spine ; fourth with a pair of strong closely placed spines ; fifth with a pair of widely spaced strong hooks, the inner of which is bifid ; sixth with a single bifid hook.

COCOON (fig. 4, *a*). 4·5 mm. in length, gelatinous and interwoven with coarse threads with a reinforced distal edge.

Simulium alcocki Pomeroy

Described from specimens collected by Dr. J. Bequaert at Elisabethville and Pania Mutombo (Kasai).

FEMALE dissected from mature pupa.

Abdomen. *Spermatheca* and *terminalia* as in *nigritarsis*.

MALE dissected from mature pupa.

Abdomen. *Terminalia* (fig. 9). *Coxite* (fig. 9, *a*) long, narrow and distinctly concave on its inner side when examined in lateral aspect. *Clasper* about as long as the coxite, and tapering gradually to the tip, which is armed with a small chitinized tooth. *Phallosome* : *anterior part* (fig. 9, *b*) about twice its distal width basally, with two inward- and downward-projecting lateral anterior processes ; in lateral view (fig. 9, *c*) part of its posterior side appears turned over, and its flat anterior end is clearly seen ; median process broad and long, with a hooked end ; *posterior part* (fig. 9, *d*) consisting of a pair of spine-like parameres similar to *bequaerti*. *Cercus* (fig. 9, *e*) long, narrow and inwardly curved distally.

LARVA unknown.

PUPA.

Head and *thorax* with disc-like tubercles and simple trichomes.

Respiratory organ (fig. 3, *d*) consisting of 7 long pale semi-translucent filaments arising from 3 stems. Pomeroy (1922) describes their arrangement as

follows : ' The first main branch divides dichotomously at some distance from base ; the second divides dichotomously and once again, making three branches in all ; the third divides dichotomously at some distance from the base, but arises in conjunction with the second rather than from the main base.' The

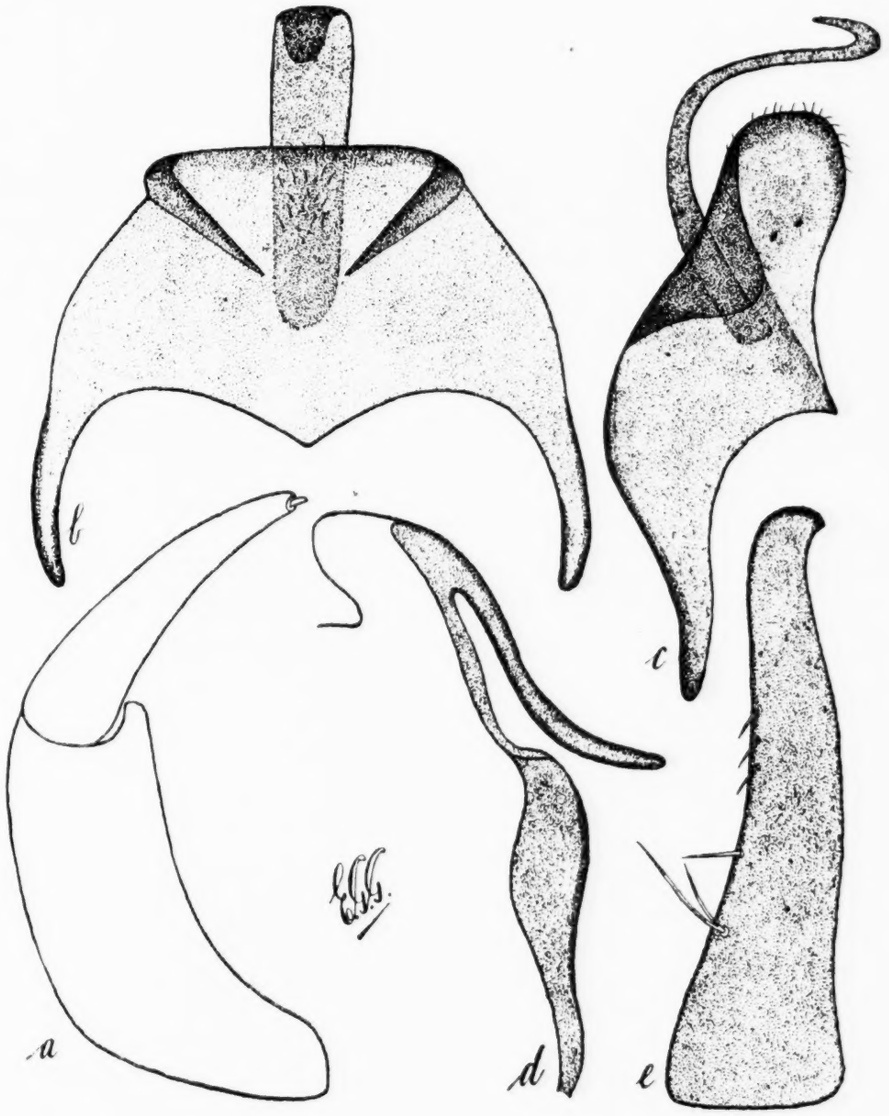


FIG. 9. *S. alcocki*. Male terminalia. *a*.—Coxite and clasper in ventral view ; *b*.—Phallosome, anterior part, ventral view ; *c*.—Lateral view ; *d*.—Right side of posterior part in dorsal view ; *e*.—Cercus. (Magnification of *a* as in fig. 1 ; of *b*, *c*, *d*, *e* as in fig. 10.)

outer wall under high magnification is seen to be covered with small nodules which are arranged in rows over the entire surface (fig. 3, *f*), and the base shows disc-like tubercles, as depicted in fig. 3, *e*.

Abdomen with a terminal pair of inwardly curved hooks. Dorso-lateral surface : third and fourth segments with a row of 4 strong, equally spaced hooks ;

eighth with a pair of closely placed downward-projecting strong spines. Ventro-lateral surface : fifth segment with a pair of strong bifid hooks which are closely placed ; sixth and seventh with a single bifid hook.

COCOON (fig. 4, *e*). 3 mm. in length, gelatinous and closely interwoven with coarse threads with an anterior median projection.

Simulium violaceum Pomeroy

(*Simulium alcocki* Pomeroy var. *violaceum* Pomeroy)

Simulium violaceum was previously described by Pomeroy (1922) as a variety of *S. alcocki*. The very marked differences in all the parts comprising the male terminalia from that of *alcocki*, as shown in the present study, appear to warrant its elevation to the status of a distinct species.

Described from specimens collected by Dr. Hissette at Elisabethville in the River Tshamulolo.

FEMALE. Length in spirit 2 mm. ; wing 1·7 mm.

Abdomen. Spermatheca (fig. 7, *e*) irregularly oval and heavily chitinized ; duct unchitinized. *Terminalia* as in *S. adersi* (Gibbins, 1934).

MALE dissected from mature pupa.

Abdomen. Terminalia (fig. 10). *Coxite* (fig. 10, *a*) broad in ventral aspect, with a short outer anterior process covered with strong bristles. *Clasper* slightly longer than the coxite, narrow and cylindrical, with a rounded end bearing a strong apical tooth. *Phallosome* : *anterior part* (fig. 10, *b*, *c*) slightly broader distally than basally, with a large conical forwardly projecting median process armed with numerous setae, lateral downward-projecting processes with inwardly curved ends; median process broad and long, with a backwardly hooked end ; *posterior part* as in *bequaerti*. *Cercus* large, with short bristles arranged below the pigmented area, as in figure 10, *d*.

LARVA unknown.

PUPA.

Head and *thorax* with disc-like tubercles and short simple trichomes.

Respiratory organ (fig. 3, *g*) of the *hirsutum* type. The 10 slender semi-translucent filaments with an outer wall, as depicted in fig. 3, *f*, are slightly greater than half the length of those of *S. alcocki* ; they arise from three main stems, which divide a short distance from the base and give rise to 2, 3 and 5 filaments respectively.

Abdomen. Terminal segment with a pair of strong terminal spines. Dorso-lateral surface : third and fourth segments with 4 closely placed strong hooks ; sixth with 3 and seventh with 4 strong, downward-projecting spines ; eighth with a row of 6 spines of irregular size ; and ninth with a basal row of 6 spines.

Ventro-lateral surface : fifth segment with a single strong slender spine ; sixth, seventh and eighth with a pair of slender bifid spines, those on the sixth segment being widely placed.

COCOON as in *alcocki*.

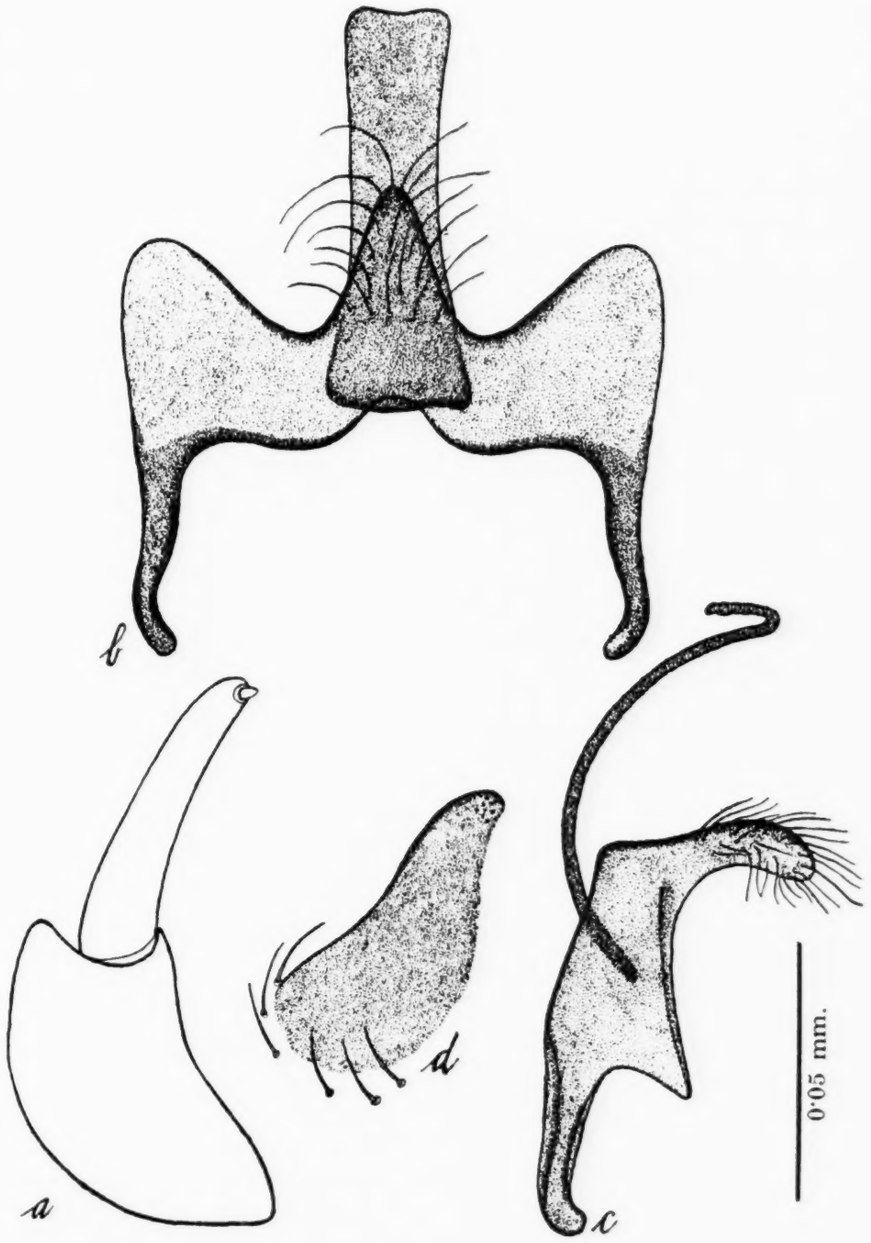


FIG. 10. *S. violaceum*. Male terminalia. *a*.—Coxite and clasper in ventral view ; *b*.—Phallosome, anterior part, ventral view ; *c*.—Lateral view ; *d*.—Cercus. (Magnification of *a* as in fig. 1.)

DISTRIBUTION OF *SIMULIUM* IN THE BELGIAN CONGO

The determinations on which the distribution is based were made by the writer either on the character of the respiratory organ of the pupa or on the terminalia of the adult. With the exception of the small collection of adults from the Congo Museum in Belgium, and of a number of specimens noted as having been caught, the flies were reared from pupae, whose cases had been preserved together with the adults. In some instances individuals had been isolated, but in the majority of cases two or more species were present in the same tube, which made it necessary, when there were closely related females inseparable on the terminalia, to base identification on the respiratory organ of the pupal pelt.

Simulium adersi Pomeroy

Described by Pomeroy (1922) as a variety of *S. hirsutum* on the character of the respiratory organ of the pupa ; raised to the status of a species by the present writer (1934), when the male, female, pupa and larva were described.

Elisabethville (Katanga), Rivers Lubumbashi, Kimilolo and Kafubu, Dr. Bequaert and Dr. Hissette ; Kundelungu Mts. (Katanga), west of Lake Moero, sources of River Katwe, Oct. 28th, 1934, Dr. J. Hissette.

Simulium alcocki Pomeroy

Male and female described by Pomeroy (1922) ; terminalia of male and female dealt with in the present study.

Elisabethville (Katanga), August, 1934, Dr. J. Bequaert ; Kilwa (Katanga), on Lake Moero (in small stream), Dr. J. Hissette ; River Musosoi, Mulubule (Kasai), 5° 20′ S., 23° 45′ E., Dr. J. Bequaert ; Pania Mutombo (Kasai), 5° 11′ S., 23° 47′ E., Dr. J. Bequaert ; Kundelungu Mts. (Katanga), west of Lake Moero, Dubie, Nov., 1934, Dr. J. Hissette ; Luputa (Kasai) (2 males, 4 females, caught in house on window panes), July, 1934, Dr. J. Bequaert.

Simulium bequaerti Gibbins

Male, female and pupa dealt with in the present study.

Kundelungu Mts. (Katanga), west of Lake Moero, sources of Mushipushi and Lutipuka, Nov., 1934, Dr. J. Bequaert ; Malela village (Chief Kasende) (Kasai), 5° 40′ S., 23° 45′ E., Dr. J. Bequaert ; Luputa (Kasai), 7° 10′ S., 23° 30′ E., Dr. J. Hissette.

Simulium cervicornutum Pomeroy

Male and female described by Pomeroy (1920); redescribed by the present writer (1936*a*), when the larva and pupa were also dealt with.

Elisabethville (Katanga), River Lubumbashi, Dr. J. Bequaert ; Mulubule (Kasai), River Musosoi, 5° 20′ S., 23° 45′ E., Dr. J. Bequaert ; Pania Mutombo (Kasai), 5° 11′ S., 23° 47′ E., Dr. J. Bequaert ; Kabinda (Kasai), River Lukumbi, 6° 8′ S., 24° 21′ E., Dr. J. Bequaert.

Simulium damnosum Theobald*

Female described by Theobald (1903) ; female redescribed and male, larva and pupa described by the present writer (1933).

No examples of *S. damnosum* were amongst the bred material ; the specimens determined were all females and had been taken as adults.

Pania Mutombo, 5° 11′ S., 15° 20′ E., July 15th, 1934, Dr. J. Bequaert ; La Panda (Katanga), 10° S., 26° 50′ E., Oct. 2nd, 1920, Michael Bequaert ; Leopoldville, 4° 25′ S., 15° 20′ E., Aug., 1911, Dr. A. Dubois.

Simulium duodecimum Gibbins

Male, female, larva and pupa described by the present writer (1936*a*).

Kabinda (Kasai), 6° 8′ S., 24° 21′ E., Dr. J. Bequaert ; Luputa (Kasai), 7° 10′ S., 23° 30′ E., Dr. J. Hissette ; Kanda Kanda (Kasai), 7° S., 23° 30′ E., Dr. Hissette.

Simulium hirsutum Pomeroy

Male and female described by Pomeroy (1922) ; both sexes were redescribed by the present writer (1936*a*), when a description of the larva and pupa was also given.

Elisabethville (Katanga), River Lubumbashi, Dr. J. Bequaert ; Pweto (Katanga), on Lake Moero, 8° 30′ S., 28° 55′ E., in rapids of River Luvua, Nov., 1934, Dr. J. Hissette ; River Musosoi, Mulubule (Kasai), 5° 20′ S., 23° 45′ E., Dr. J. Bequaert ; Pania Mutombo (Kasai), 5° 11′ S., 23° 47′ E., Dr. J. Bequaert ; Kundelungu Mts. (Katanga), west of Lake Moero, Dubie, Nov., 1934, Dr. J. Hissette.

Simulium hissetteum Gibbins

Male, female and pupa dealt with in the present study.

Luputa (Kasai), 7° 10′ S., 23° 30′ E., Dr. J. Hissette ; Kanda Kanda (Kasai), River Luilu, 7° S., 23° 30′ E., Dr. J. Hissette.

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*Hitherto *Simulium damnosum* appears to be the only species of *Simulium* that may be definitely associated with the Belgian Congo. Bequaert (1930) records the species from : Matada, Dec. 4th, 1926 ; Leopoldville, Dec., 1926 ; River Uele, between Bambili and Angu, J. Rodhain ; Matada, Lutete, Leopoldville, near Miambwe, Newstead, Dutton and Todd ; River Uele, Brumpt ; Djamba on the River Itimbiri, Schouteden.

Simulium kauntzeum Gibbins

Male, female, larva and pupa described by the present writer (1936*b*).

Mt. Ruwenzori, western slope, upper Butahu Valley, at 4,200 m. (pinned female from Congo Museum), July 2nd, 1932, L. Burgeon.

Simulium neavei Roubaud

Female described by Roubaud (1915).

Village of Malela (Chief Kasende), 5° 40′ S., 23° 45′ E., Dr. J. Bequaert (pinned females).

Simulium nigritarsis Coquillett

Female described by Coquillett ; female redescribed and male described by De Meillon (1930) ; respiratory organ of the pupa and male terminalia were dealt with by the same author (1934). Male and female terminalia fully described by the writer in the present study.

Elisabethville (Katanga), Rivers Tshamulolo and Lubumbashi, Dr. J. Hissette ; Kilwa (Katanga), on Lake Moero (in small stream), 9° 20′ S., 28° 25′ E., Dr. J. Hissette ; Kanda Kanda (Kasai), River Luilu, 7° S., 23° 30′ E., July, 1934, Dr. J. Hissette ; Lugenga (north of Lake Kivu), 1,800 m., 1° 20′ S., 29° 20′ E. (single pinned specimen), Nov. 30th, 1925, Dr. H. Schouteden.

Simulium ruficorne Macquart

Edwards (1934) has shown that *S. beckeri* Roubaud, *S. diversipes* Edwards (1922) and *S. divergens* Pomeroy (1922) are synonyms of *S. ruficorne*. Male, female, larva and pupa redescribed by the present writer (1936*a*).

Luanza (Katanga), on Lake Moero, 8° 45′ S., 28° 45′ E., Dr. J. Hissette ; Pweto (Katanga), on Lake Moero, River Luvua, 8° 30′ S., 28° 55′ E., Nov., 1934, Dr. J. Hissette ; Kundelungu Mts. (Katanga), west of Lake Moero, sources of River Luanza, Nov., 1934, Dr. J. Hissette ; Kabinda (Kasai), River Lukumbi, 6° 8′ S., 24° 21′ E., Dr. J. Bequaert ; Kanda Kanda (Kasai), River Luilu, 7° S., 23° 30′ E., Dr. J. Hissette.

Simulium ruwenzoriensis Gibbins

Male, female, larva and pupa described by the present writer (1934).

Mt. Ruwenzori, Batahu valley, 2,000 m., May 14th, 1914 (single pinned female*), Dr. J. Bequaert.

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*This specimen came from the Congo Museum in Belgium and was previously determined as *Simulium dentulosum* (see Bequaert, 1930). A critical examination of its dissected terminalia, however, proved that it was no other than *Simulium ruwenzoriensis*.

Simulium tentaculum Gibbins

Male, female, larva and pupa described by the present writer (1936*a*).

Lukonzolwa (Katanga), on Lake Moero, 8° 50′ S., 28° 40′ E., Dr. J. Hissette ; Kundelungu Mts. (Katanga), west of Lake Moero, source of River Lutipuka, Nov., 1934, Dr. J. Hissette ; Luanza (Katanga), Lake Moero, 8° 50′ S., 28° 45′ E., Nov., 1934, Dr. J. Hissette.

Simulium ugandae Gibbins

Male, female, pupa and larva described by the present writer (1934).

Elisabethville, River Lubumbashi, Dr. J. Bequaert and Dr. Hissette ; Lukonzolwa (Katanga), on Lake Moero, 8° 50′ S., 28° 40′ E., in small stream 3 Km. north of Lukonzolwa, Nov., 1934, Dr. J. Hissette ; Luanza (Katanga), on Lake Moero, River Luanza, 8° 45′ S., 28° 45′ E., Nov., 1934, Dr. J. Hissette ; Kundelungu Mts. (Katanga), west of Lake Moero, sources of River Katwe and River Mushipushi, Oct., 1934, Dr. J. Hissette.

Simulium unicornutum Pomeroy

Described by Pomeroy (1920) on the character of the respiratory organ of the pupa ; male and female described by the same author (1922) and redescribed by the present writer (1936*a*), when the larva and pupa was also dealt with.

Mulubule (Kasai), River Musosoi, 5° 20′ S., 23° 45′ E., Dr. J. Bequaert ; Pania Mutombo (Kasai), 5° 11′ S., 23° 47′ E., Dr. J. Bequaert.

Simulium violaceum Pomeroy

Described by Pomeroy (1922) as a variety of *S. alcocki* on the character of the respiratory organ of the pupa ; raised to the status of a species in the present study. Male and female terminalia and pupa described.

Elisabethville (Katanga), River Tshamulolo, Dr. J. Hissette ; Lukonzolwa (Katanga), on Lake Moero, 8° 50′ S., 28° 40′ E., Nov., 1934, Dr. J. Hissette ; River Musosoi, Mulubule (Kasai), 5° 20′ S., 23° 45′ E., Dr. J. Bequaert ; Pania Mutombo (Kasai), 5° 11′ S., 23° 47′ E., Dr. J. Bequaert.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS GLOSSINA WIEDEMANN BASED ON A COMPARATIVE STUDY OF THE MALE AND FEMALE TERMINALIA

(*Continued from page* 89)

BY

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Glossina medicorum Austen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A medium-sized (♂) to fairly large species (♀), with rather ill-defined pale thoracic markings. Third segment of antenna (fig. 1) with rather prominent end, and branches of aristal hairs very short. Wings pale, the veins rather distinctly yellowish brown. Abdomen dark brown.

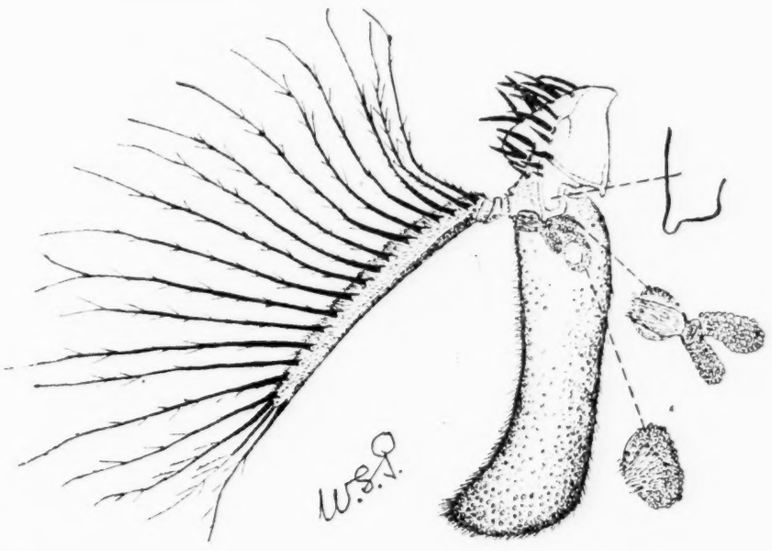


FIG. 1. Antenna of ♂ *medicorum* with peg and sensoria shown enlarged.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Fig. 2. Long, stout, rod-like plates, with a strong pointed process on inner side of end. *Phallosome.* Fig. 3, *a.* The distal part long, with a broad, ventral, hood-like plate with a pair of long dorsal processes on each side of the membraneous end ; apodeme of phallosome with a marked dorsal projection. Paramere and median

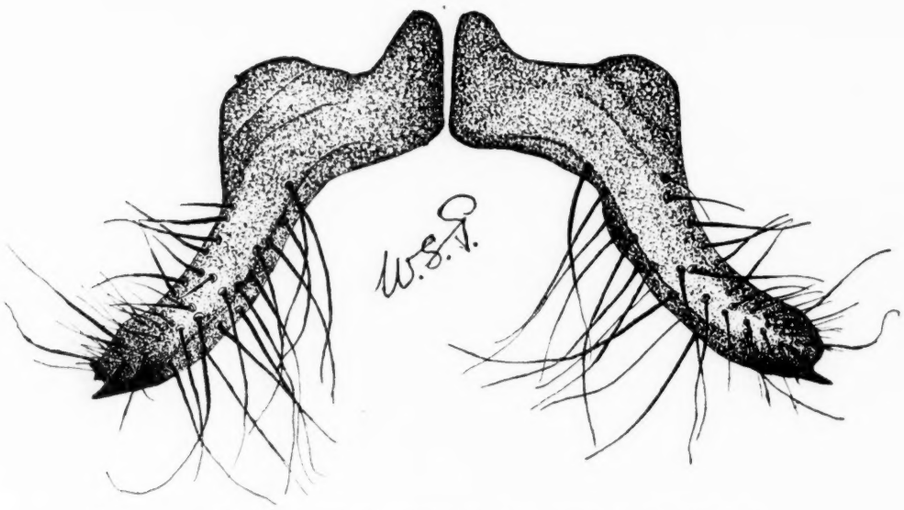


FIG. 2. Ventral view of extended anal cerci of *medicorum*.

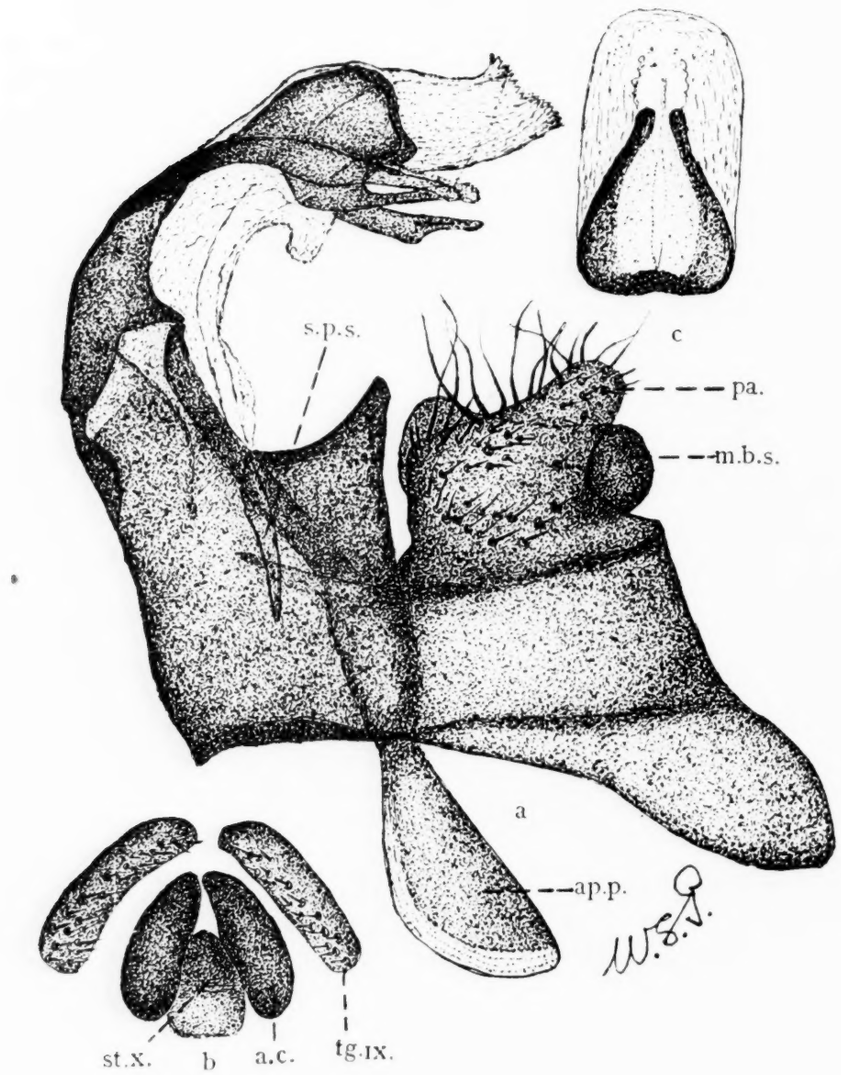


FIG. 3. *a.*—Lateral view of ninth tergo-sternum of *medicorum* showing phallosome embedded in its dorsal concavity ; *ap.p.*—apodeme of phallosome ; *m.b.s.*—median binding sclerite ; *p.a.*—paramere ; *s.p.s.*—sperm pump sclerite ; *b.*—Terminalia of ♀ *medicorum* ; *a.c.*—anal cercus ; *st.x.*—tenth sternum ; *tg.ix.*—ninth tergum ; *c.*—Signum.

binding sclerite as in fig. 3, *a*. ♀. Fig. 3, *b*. Ninth tergal plates rather long and narrow, as are also the anal cerci. Signum of a characteristic shape, as shown in fig. 3, *c*.

DISTRIBUTION. HABITS. IMPORTANCE. *G. medicorum* is a West African species and has been recorded from Dahomey, Gold Coast, Ivory Coast, Ashanti and Southern Nigeria; one specimen is recorded from Principe Island. In northern Ashanti Ingram found it in savannah forest. Practically nothing is known of its habits. It is not an important carrier of trypanosomes.

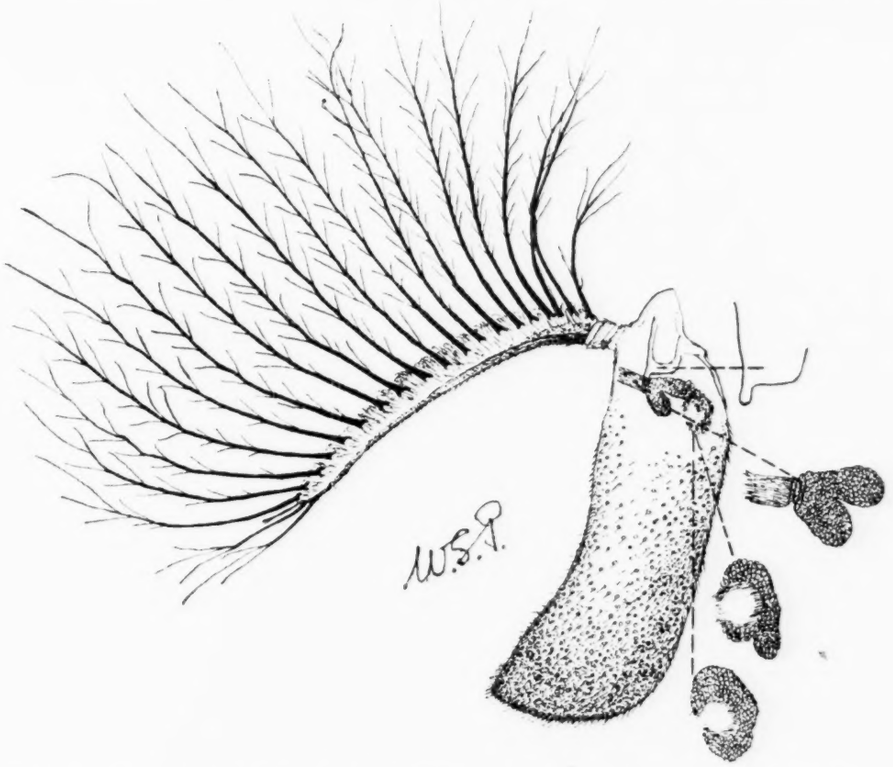


FIG. 4. Antenna of ♂ *haningtoni* with peg and sensoria shown enlarged.

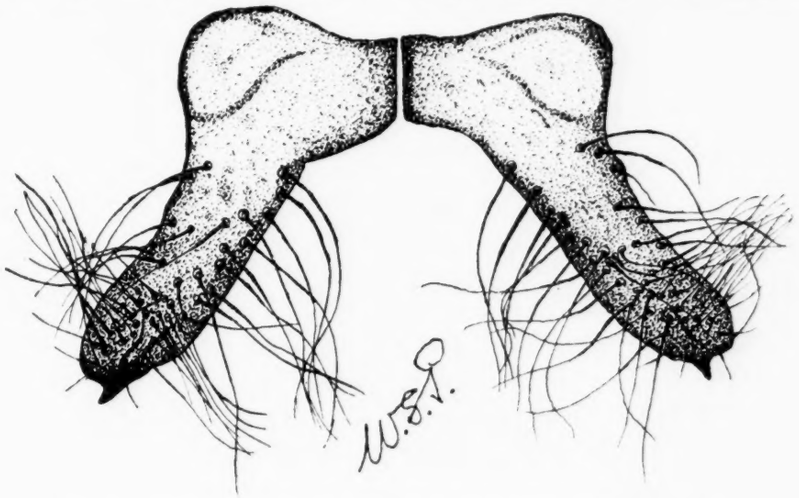


FIG. 5. Ventral view of extended anal cerci of *haningtoni*.

Glossina haningtoni Newstead and Evans. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A large dark species with infuscated wings. Third segment of antenna (fig. 4) broad and somewhat pointed at end, the arista with long hairs and branches. Thoracic markings dark. Abdomen dark brown.

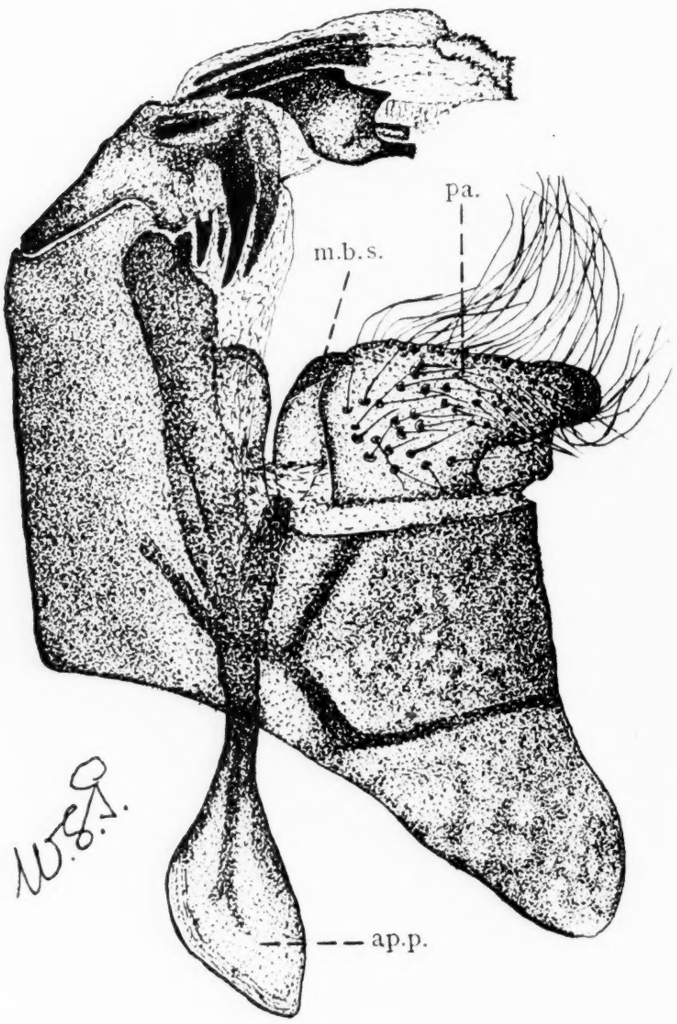


FIG. 6. Lateral view of ninth tergo-sternum of *haningtoni* showing phallosome embedded in its dorsal concavity; lettering as in fig. 3, *a*.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Figs. 5; 7, *b*. Rather short wide plates, with a small pointed process on inner side, the outer side rounded. *Phallosome.* Figs. 6; 7, *a*. Distal part with a pair of ventral pointed processes near base, close to where it articulates with the apodeme, one longer than the other. A curved plate on each side of distal end shaped

as shown in figs. 6 ; 7. Paramere (fig. 6) rather long, the end projecting freely ; median binding sclerite not prominent. ♀. Fig. 7, *d*. Ninth tergal plates long. Signum as in fig. 7, *d*.

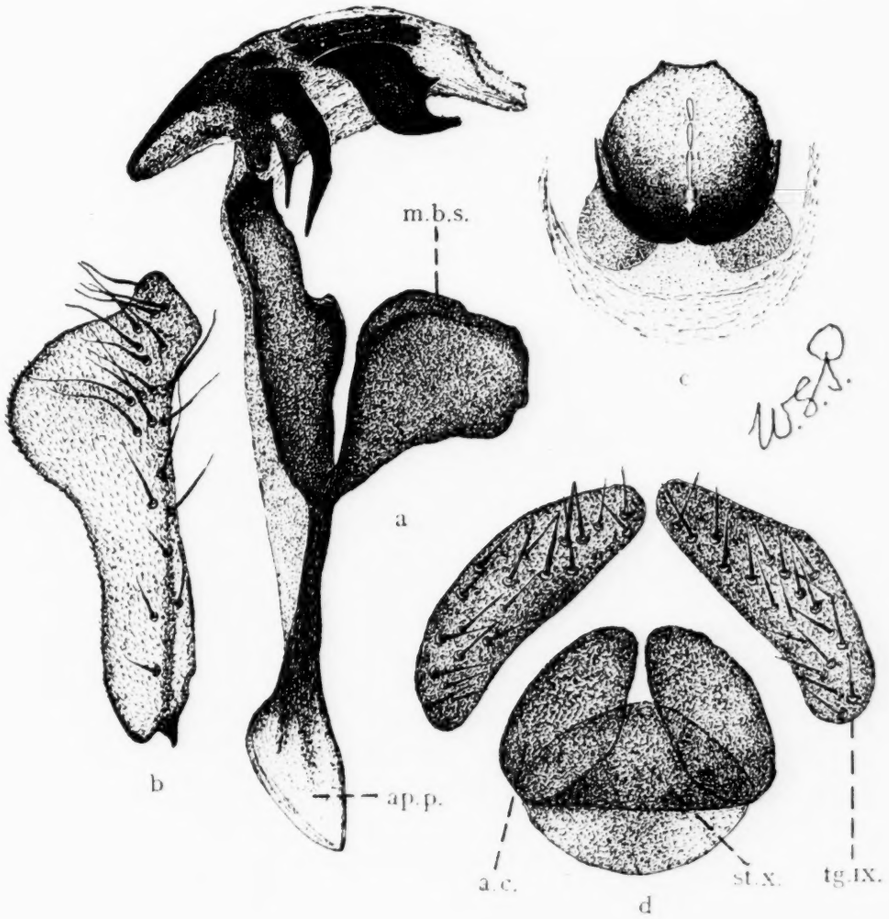


FIG. 7. *a*.—Lateral view of phallosome of *haningtoni* dissected out of ninth tergo-sternum ; *ap.p.*—apodeme of phallosome ; *m.b.s.*—median binding sclerite ; *b*.—Dorsal view of anal cercus of same ; *c*.—Signum ; *d*.—Terminalia of ♀ *haningtoni* ; lettering as in fig. 3, *b*.

DISTRIBUTION. HABITS. IMPORTANCE. This is also mainly a West African species, though Hegh has recently recorded it from Mayumbe in the Katanga Province of south-eastern Belgian Congo. Hanington, the discoverer of the species, found it in the south Cameroons in two localities ; Galliard has recently recorded having collected one specimen from the western Gabon. It is mainly found in forests bordering rivers. Nothing is known of its habits or its importance.

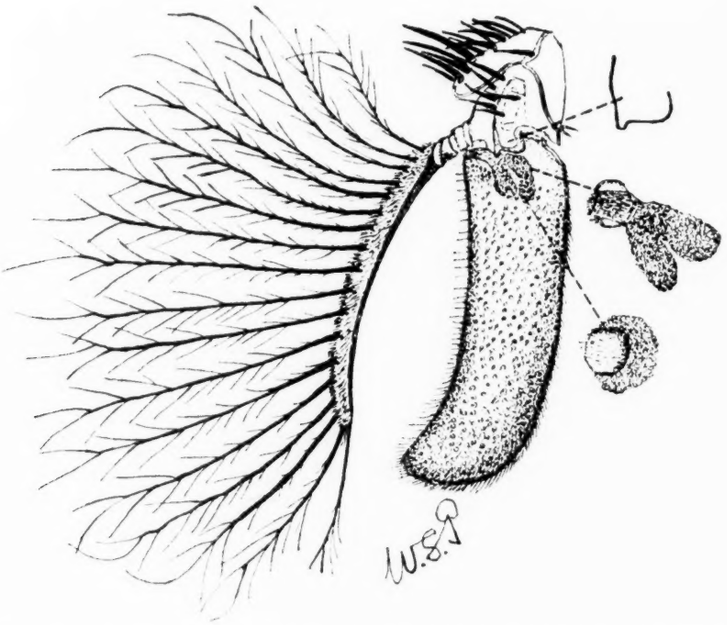


FIG. 8. Antenna of ♂ *schwetzi* with peg and sensoria enlarged.

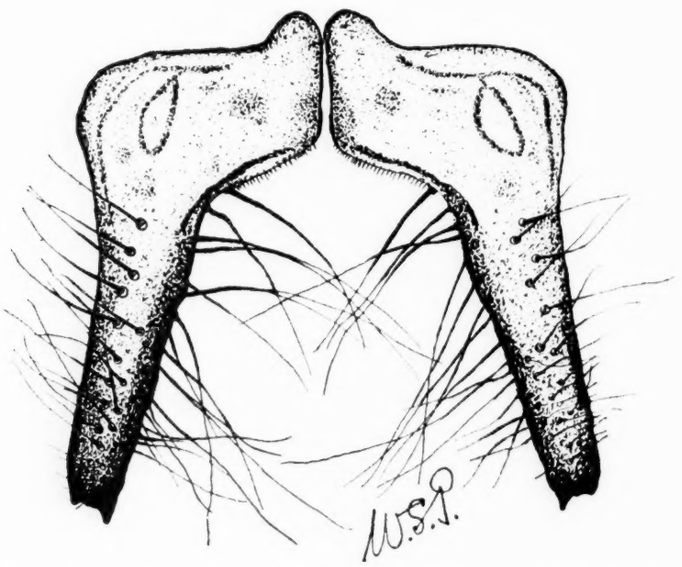


FIG. 9. Ventral view of extended anal cerci of *schwetzi*.

Glossina schwetzi Newstead and Evans. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A moderately large species resembling *tabaniformis* and *brevipalpis*. Third segment of antenna (fig. 8) directed forwards and pointed at its distal end ; and hairs on its anterior border, although long, are distinctly shorter than those of *tabaniformis* ; aristal hairs and their branches long. In all other respects this species is very like the two mentioned above.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Figs. 9; 10, *b*. Long stout plates, with a short rather blunt process on inner side, and the suggestion of a process on the outer side as well. *Phallosome.* Figs. 10; 11. Rather short, especially the distal portion, which has three processes on each side; the basal pair are part of the ventral plate, and the distal one is the

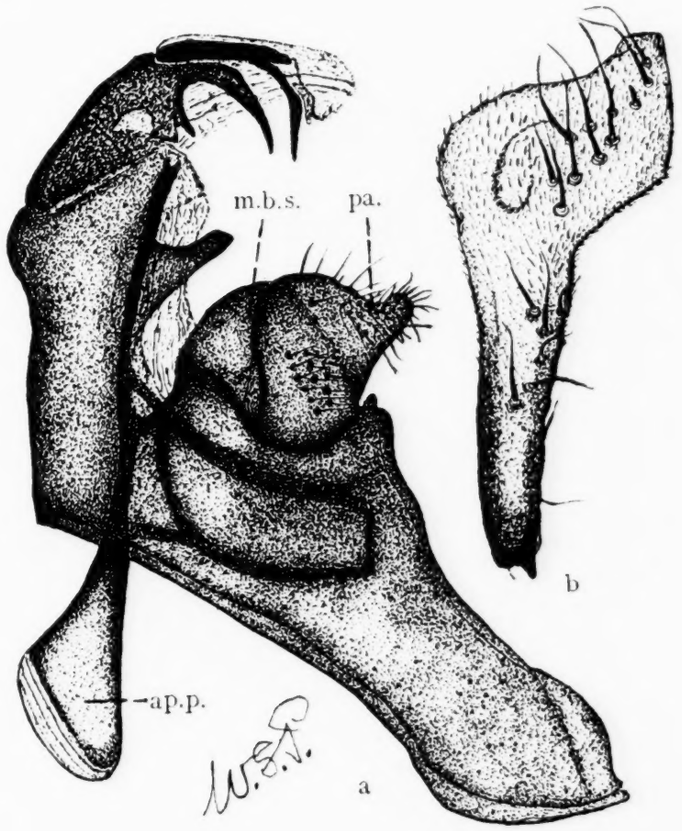


FIG. 10. *a*.—Lateral view of ninth tergo-sternum of *schwetzi* showing phallosome embedded in its dorsal concavity; lettering as in fig. 3, *a*; *b*.—Dorsal view of anal cercus.

longer; the third process is separate; there is also a pair of median plates on the ventral side at the distal end. These appearances are well seen in the profile illustrations in figs. 10 and 11. The dorsal view of the end of the abdomen is shown in fig. 11, *b*. ♀. Fig. 11, *d*. The ninth tergal plates are long, and the anal cerci are small. The signum is illustrated in fig. 11, *c*.

DISTRIBUTION. HABITS. IMPORTANCE. *G. schwetzi* is only known from the Belgian Congo, where it has been recorded from the following localities: Kwango River, Lasongo Lunda; near Cuango; near Kundi; Popokohaka; between Matadi and Thysville, Congo Railway; Sukuku; Nelikagi, Luibi River, Region de Melela; Congo da Lemba; Makaia N'tete, Mayumbo. According to Schwetz, it is like *brevipalpis* in that its maximum activity begins

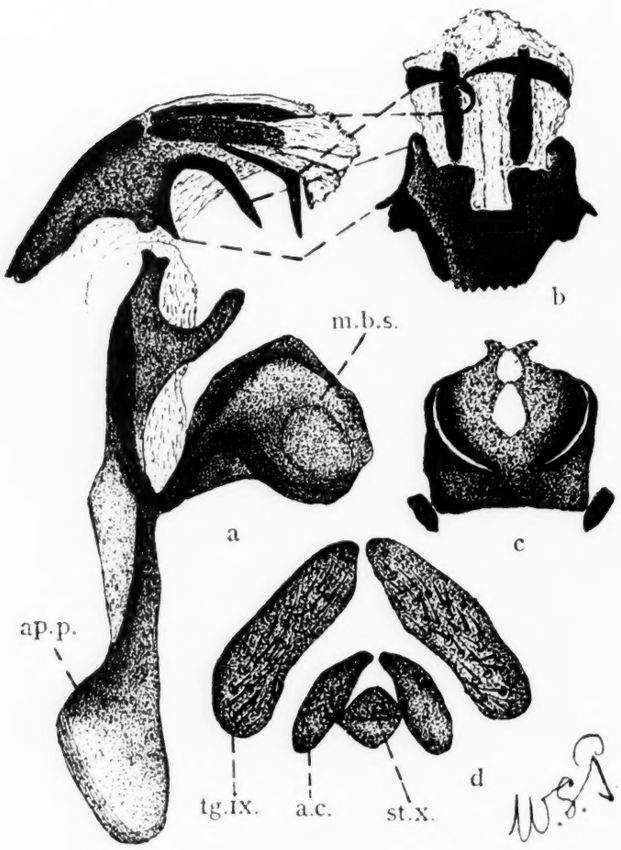


FIG. 11. *a.*—Lateral view of phallosome of *schwetzi* dissected out of ninth tergo-sternum ; lettering as in fig. 7, *a* ; *b.*—Dorsal view of end of phallosome showing the processes ; *c.*—Signum ; *d.*—Terminalia of ♀ *schwetzi* ; lettering as in fig. 3, *b.*

before and continues until after sunset. It is sometimes active during the night, like *tabaniformis*. Nothing is known of its importance as a transmitter of trypanosomes. I have not been able to examine any specimen of *Glossina schwetzi* var. *disjuncta* Potts.

Glossina severini Newstead. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A moderately large species with dusky wings. Third segment of antenna (fig. 12) long, pointed and turned forwards at the end ; arista long and stout, with long hairs and branches. Hind tarsi either dark brown or with first and second segments slightly paler than the rest. Pleurae and hind coxae dark grey.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Fig. 14, *c.* Long stout plates with a blunt process on inner side and with a rounded projection on outer side, a deep emargination between the two. *Phallosome.* Figs. 13 ; 14. Short, the distal end consisting of a single large hood-like plate deeply emarginate, the sides ending in broad points ; a small median chitinous process serrated at the end and situated on the dorsal side above the opening of

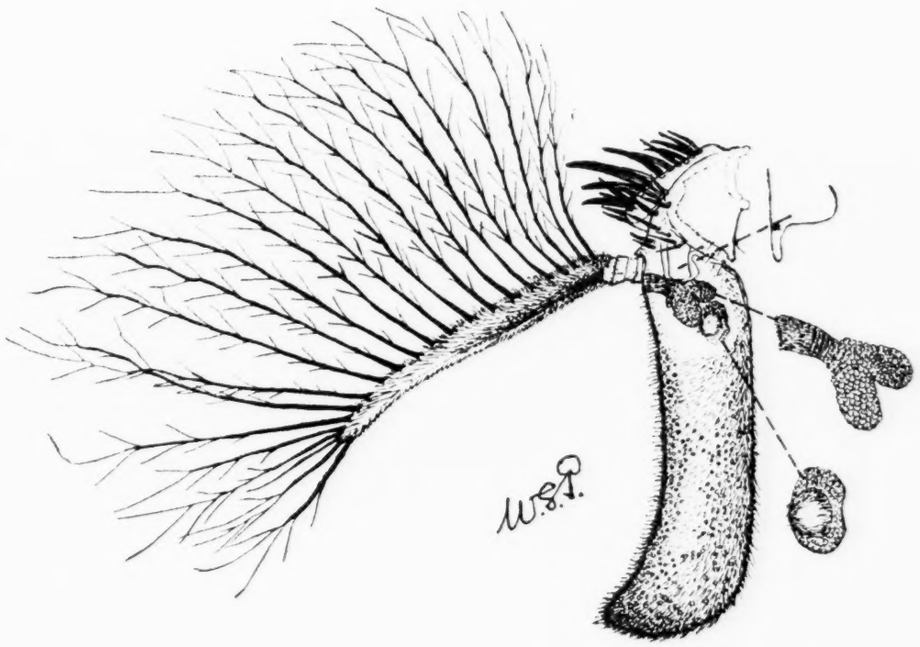


FIG. 12. Antenna of ♂ *severini* with peg and sensoria shown enlarged.

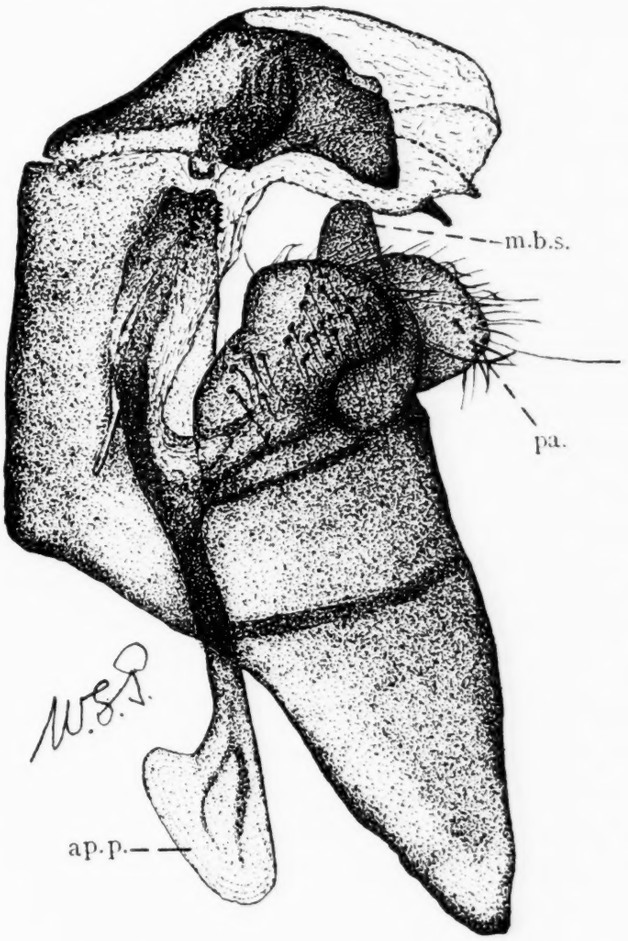


FIG. 13. Lateral view of ninth tergo-sternum of *severini* showing phallosome embedded in its dorsal concavity ; lettering as in fig. 3, *a*.

the ejaculatory duct. The median binding sclerite forms a conspicuous object projecting between the parameres (fig. 14, *a*). ♀. Fig. 14, *d*. The ninth tergal plates are large and have the characteristic shape as shown in fig. 14, *d* ; the anal cerci are relatively long and narrow. The signum is illustrated in fig. 14, *b*.

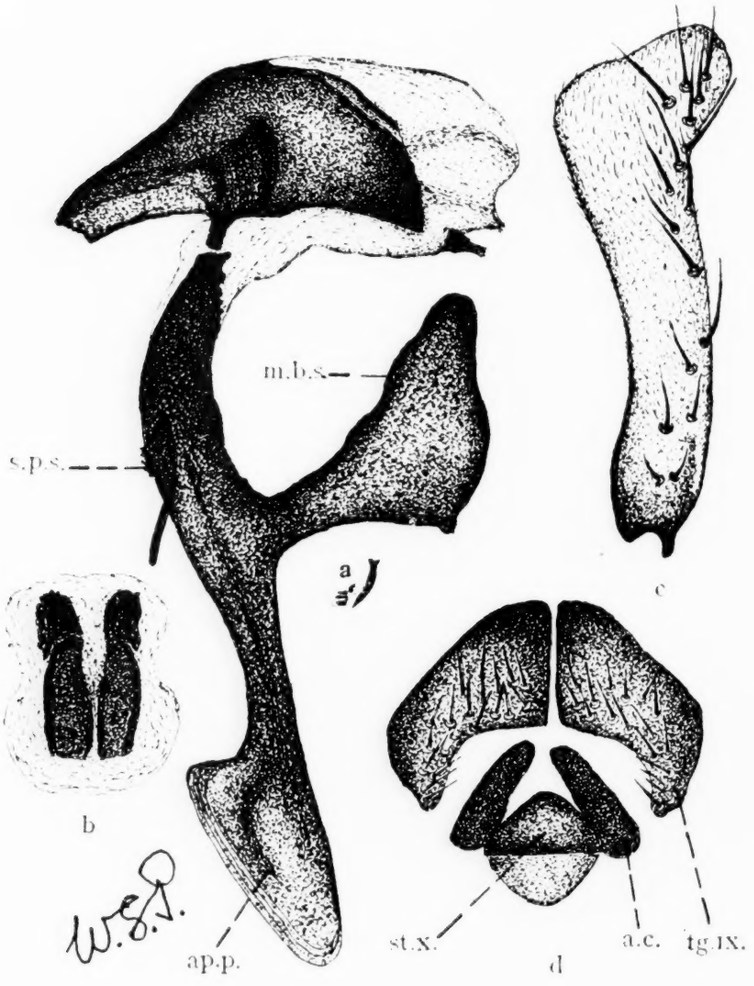


FIG. 14. *a*.—Lateral view of phallosome of *severini* dissected out of ninth tergo-sternum ; lettering as in fig. 3, *a* ; *b*.—Signum ; *c*.—Dorsal view of anal cercus ; *d*.—Terminalia of ♀ *severini* ; lettering as in fig. 1, *b*.

DISTRIBUTION. HABITS. IMPORTANCE. *G. severini* is a rare species and is known only from the Belgian Congo, where it has been taken in the Forest of Ituri, the Semliki Valley and from Zambo. Nothing is known either of its habits or of its importance.

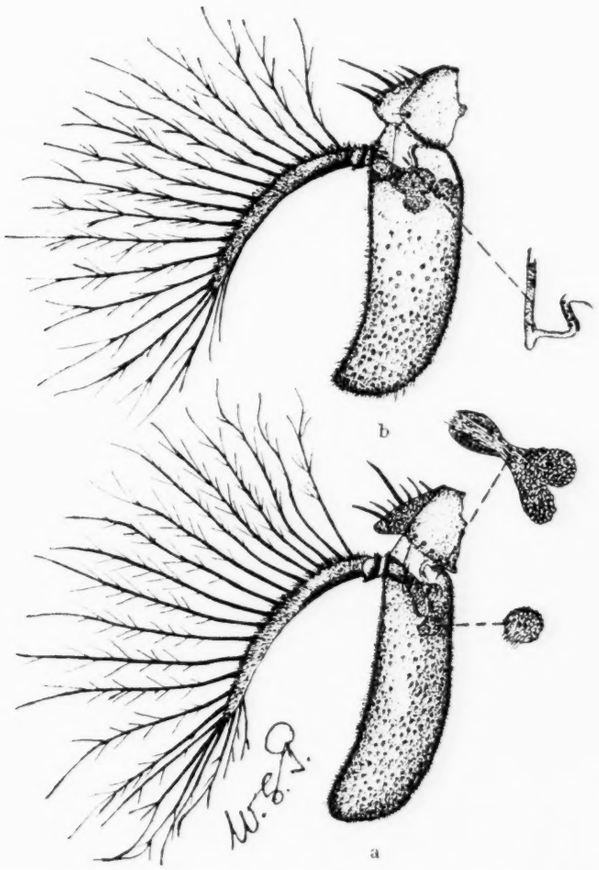


FIG. 15. *a*.—Antenna of ♂ *morsitans*; *b*.—antenna of ♀ of same; peg and sensoria shown enlarged.

SPECIES OF THE MORSITANS GROUP

Glossina morsitans Westwood. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A small light-grey species, the abdominal terga with well-marked brown bands interrupted in the middle. Third segment of antenna (fig. 15) with short hairs on its anterior border; aristal hairs with rather long branches. It will be noted that the third segment of the antenna of the ♀ (fig. 15, *b*) is bent forwards at its end slightly more than that of the ♂ (fig. 15, *a*). Thorax usually grey with darker markings. The last two segments of hind tarsi dark brown, and the last two segments of fore tarsi and the last segment of the middle tarsi brown. The brown bands on the abdominal terga though variable are conspicuous, and their inner margins are as a rule not square-edged.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. The distal segment of the ninth coxite (editum) is quite well developed in the species of this group; it consists of a small plate with long hairs at its distal end and is attached to the inner lateral margin of the tenth tergum about the middle. I shall illustrate and refer to it when considering the relationships of the species to each other.

Anal Cerci. Fig. 17, *c*, *d*. Flat, expanded plates completely joined together by membrane ; a pair of semi-membraneous lobes (fig. 17, *b*, *c*) on median border, with apices turned outwards ; numerous long hairs on inner margin of each cercus, these becoming shorter along postero-internal border (fig. 17, *c*) ; a prominent hair about middle of cercus on ventral border. *Phallosome.* Figs. 18, *a* ; 19, *b*. Short, especially the distal end which bears the opening

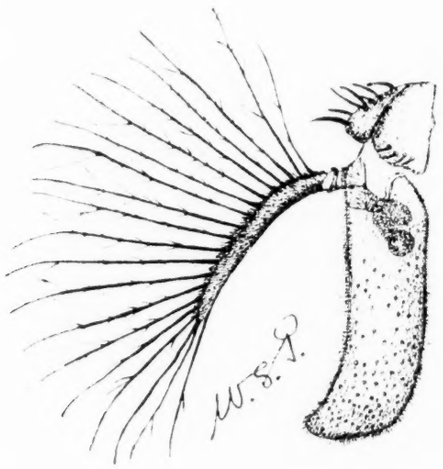


FIG. 16. Antenna of ♀ *morsitans* var. *submorsitans*.

of the ejaculatory duct. As its structure is well shown in the drawings it does not call for any description. At junction of ninth tergo-sternum with phallosome there is on each side a characteristic, long, spined, finger-like process ; it is present in all the species of this group. There is no marked dorsal projection on the apodeme of the phallosome. The sperm pump sclerite in the species of this group, as well as in those of the *palpalis* group, lies in a hollow at the distal end of the apodeme in the middle line. *Paramere.* Fig. 18, *b*. The paramere in the species of this group is saucer-shaped ; on the inner side at the distal end (which appears raised in profile, fig. 18, *a*) it is folded over, and there is a projection at the end ; along this border under the fold there are many small as well as several (the number not constant) long sensory hairs. On the outer side in the hollow there is a group of many very long hairs.

♀. The end of the abdomen of the ♀ *morsitans* and the terminalia are illustrated in fig. 20, *a*. In the species of this group the ninth tergal plates are very poorly developed, and are as a rule almost entirely membraneous; but they can be made out if the specimen is softened in 2 per cent. cold caustic potash. The anal cerci appear to be fused across the middle line dorsally, but I suspect that the middle portion is most probably the tenth tergum ; the tenth sternum is well developed ; it varies in size and shape in different specimens.

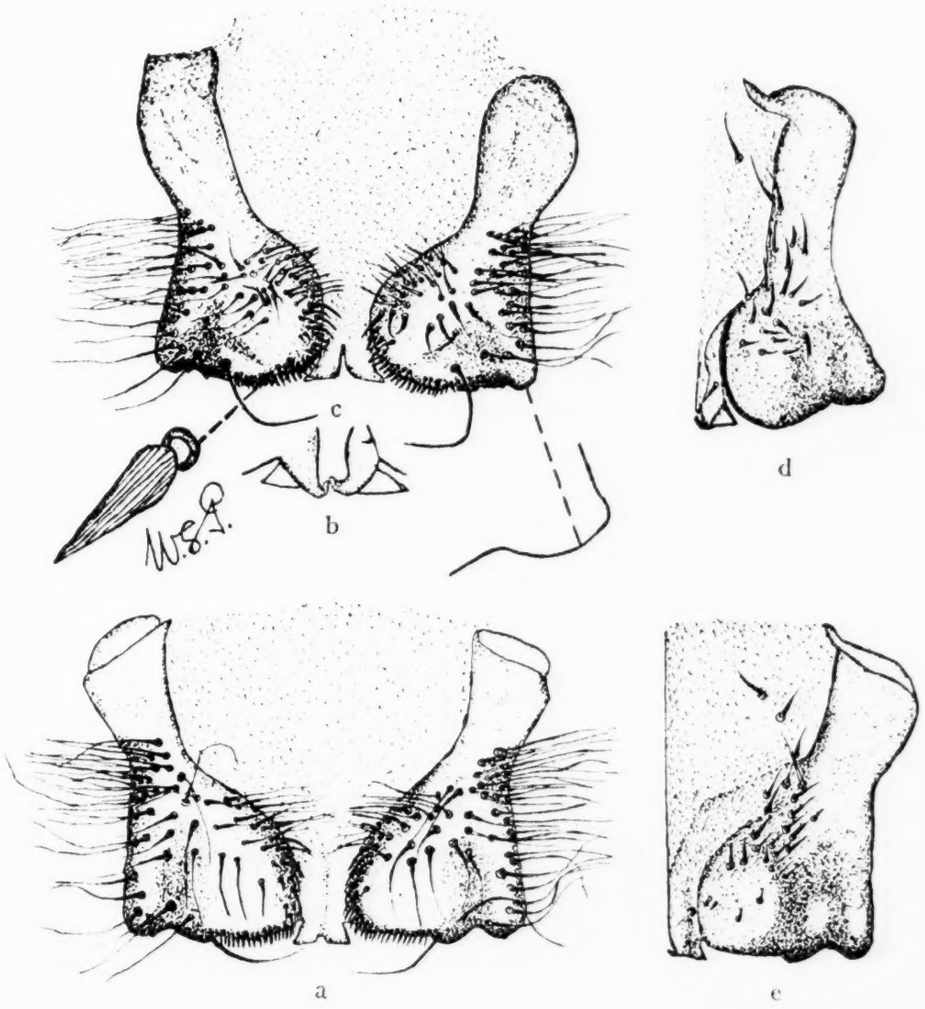


FIG. 17. *a.*—Ventral view of extended anal cerci of *morsitans* var. *submorsitans* ; *b.*—Dorsal view of median lobes of *morsitans* ; *c.*—Ventral view of extended anal cerci of *morsitans* showing one of the bristles of the inner border enlarged ; *d.*—Dorsal view of one anal cercus of *morsitans* ; *e.*—Dorsal view of one anal cercus of *submorsitans*.

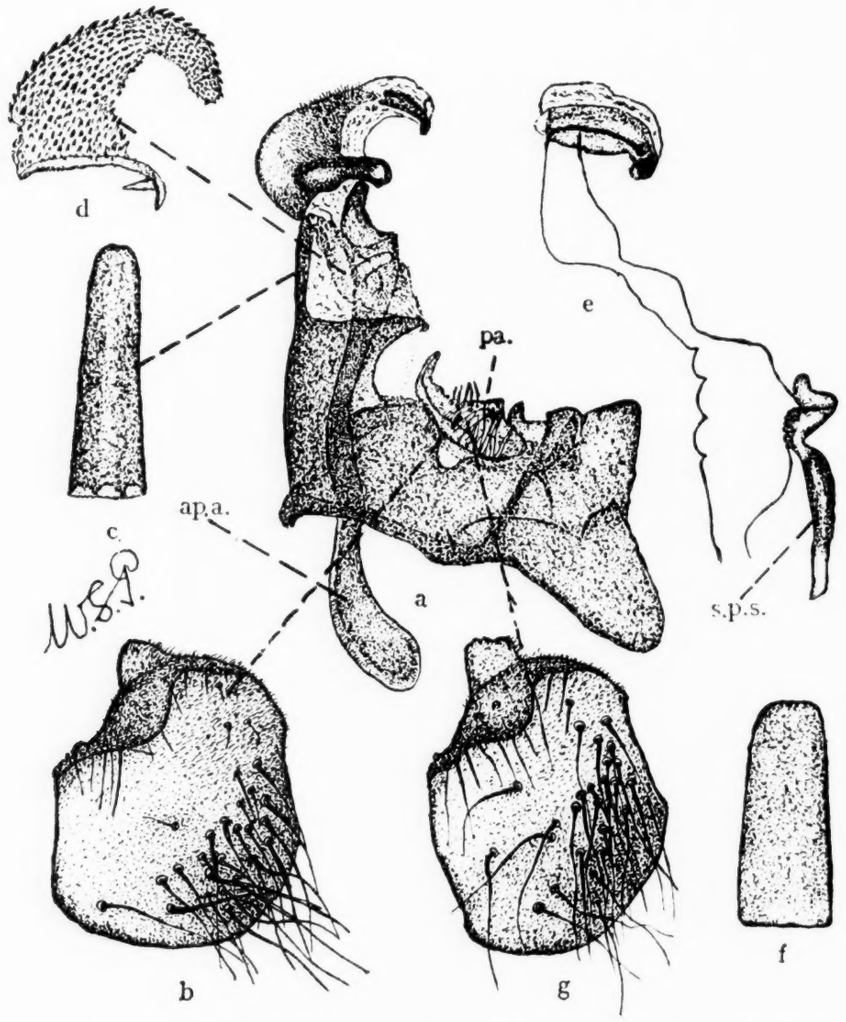


FIG. 18. *a.*—Lateral view of ninth tergo-sternum of *morsitans* showing phallosome embedded in its dorsal-concavity ; *b.*—Left paramere of *morsitans* ; *c.*—Plate on posterior wall of ninth tergo-sternum of *morsitans* against which the phallosome articulates ; *d.*—Spined membraneous finger-like flap at junction of apodeme of phallosome and ninth tergo-sternum ; *e.*—Sperm pump sclerite, ejaculatory duct and end of phallosome ; *f.*—Plate on posterior wall of ninth tergo-sternum of *morsitans* var. *submorsitans* ; *g.*—Left paramere of *morsitans* var. *submorsitans*.

DISTRIBUTION. HABITS. IMPORTANCE. *G. morsitans* is perhaps the most widely distributed species of *Glossina*. It extends from about 17° N. in the west (as the variety *submorsitans*, see below) across the continent to Kardofan in the Sudan and southern Abyssinia. It is widely distributed in the Gabon, the Middle Congo and Ubengi in French Equatorial Africa, extending into the Lake Chad region, where its distribution, however, is not continuous. It has been recorded from Angola and from many parts of the savannah forests in the Belgian Congo ; in parts of Uganda, such as South Ankole ; Masindi ; Victoria Nile, etc. ; in many localities in the western parts of Tanganyika ; in North and South Rhodesia ; in parts of Nyasaland and in Portuguese East Africa. It is doubtful if it occurs in either Kenya or Zululand. As there is a very extensive

literature dealing with this species, it would be impossible for me in the short space at my disposal to summarize any of the observations which have recently been made on its habits, habitat, etc. As is well known, this species and its Western variety *submorsitans* can live in complete independence of water. It prefers savannah and park-like country, plains with long grass or scattered shady trees, open thickets, etc. In the coastal districts it is found in thicker brushwood. It is *par excellence* the big-game fly. Next to *palpalis* it is the most important species, as it is the transmitter of pathogenic trypanosomes to man and animals.

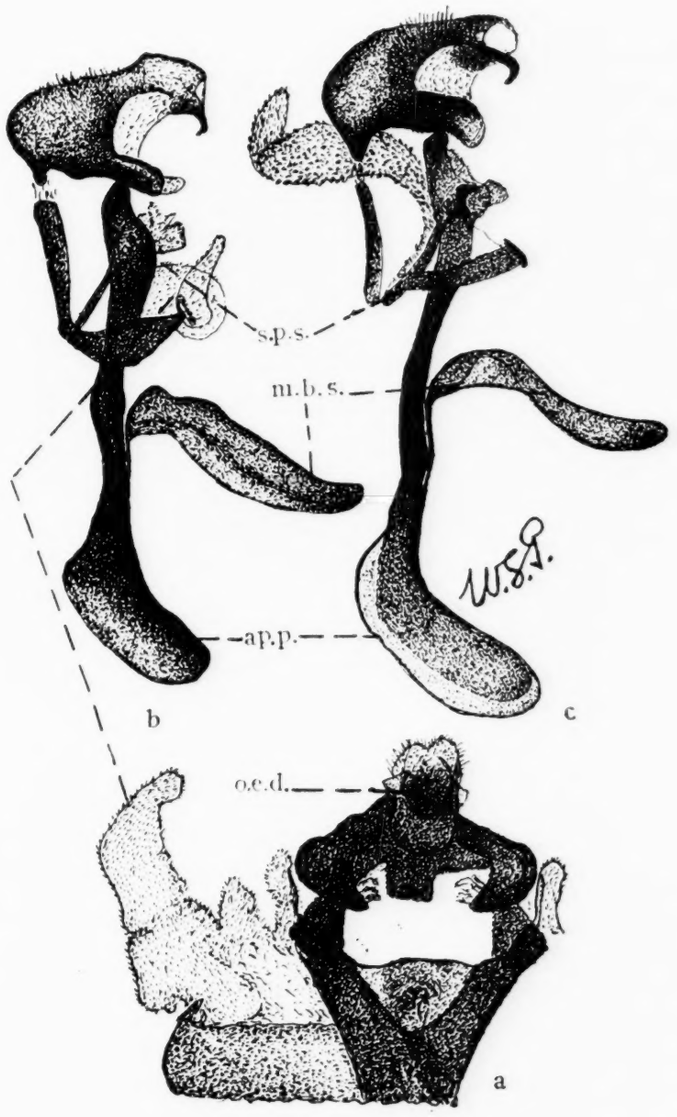


FIG. 19. *a*.—Dorsal view of end of phallosome of *morsitans* showing the spined finger-like flap on one side ; *o.e.d.*—opening ejaculatory duct ; *b*.—Phallosome of *morsitans* dissected out of ninth tergo-sternum ; lettering as in fig. 3, *a* ; *c*.—Phallosome of *morsitans* var. *submorsitans* dissected out of ninth tergo-sternum.

Glossina morsitans var. **submorsitans** Newstead. After a comparative study of the external characters of the adults and the ♂ and ♀ terminalia of this Western form of *morsitans*, I have come to the conclusion that it can only be regarded as a variety of *morsitans*, and not as a distinct species. I have been unable to find any constant characters in the terminalia which can support the view that it is a distinct species. There are, in addition, two colour varieties of *morsitans*, the pale form (*pallida* Shircore) and the dark form (*paradoxa* Shircore). Here again the comparative study of the ♂ terminalia does not justify their being considered even distinct varieties.

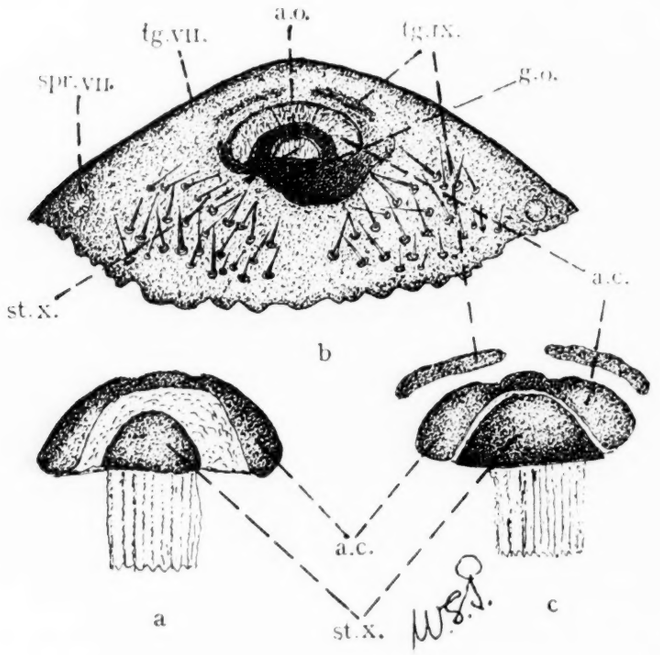


FIG. 20. *a*.—Terminalia of ♀ *morsitans* dissected out ; lettering as in fig. 3, *b* ; *b*.—End of abdomen of ♀ *morsitans* to show terminalia ; lettering as in fig. 3, *b* ; *c*.—Terminalia of ♀ *morsitans* var. *submorsitans* dissected out ; lettering as in fig. 3, *b*.

The important differences between *morsitans* and *submorsitans* according to Newstead are to be noted in the structure of the median lobes of the anal cerci. In *submorsitans* their inner margins are said to be parallel throughout their length, whereas in *morsitans* they are divergent ; I must admit that I have been unable to satisfy myself that these characters are constant.

DISTRIBUTION. HABITS. IMPORTANCE. *Submorsitans* is found throughout the western side of Africa extending into the Belgian Congo and the Sudan, but how far I do not know for certain. Its habits and habitat are similar to those of *morsitans*. It is an important transmitter of trypanosomes to animals.

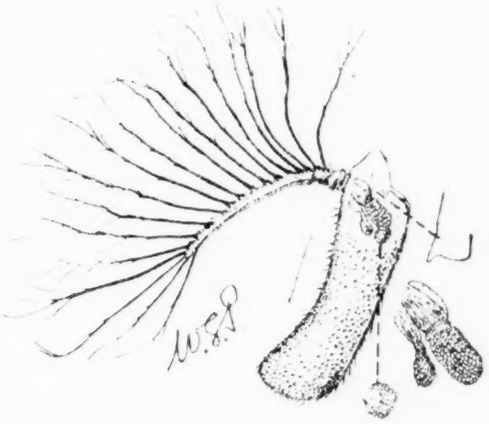


FIG. 21. Antenna of ♂ *swynnertoni* with peg and sensoria shown enlarged ; this antenna is drawn to the same scale as all the others illustrated.

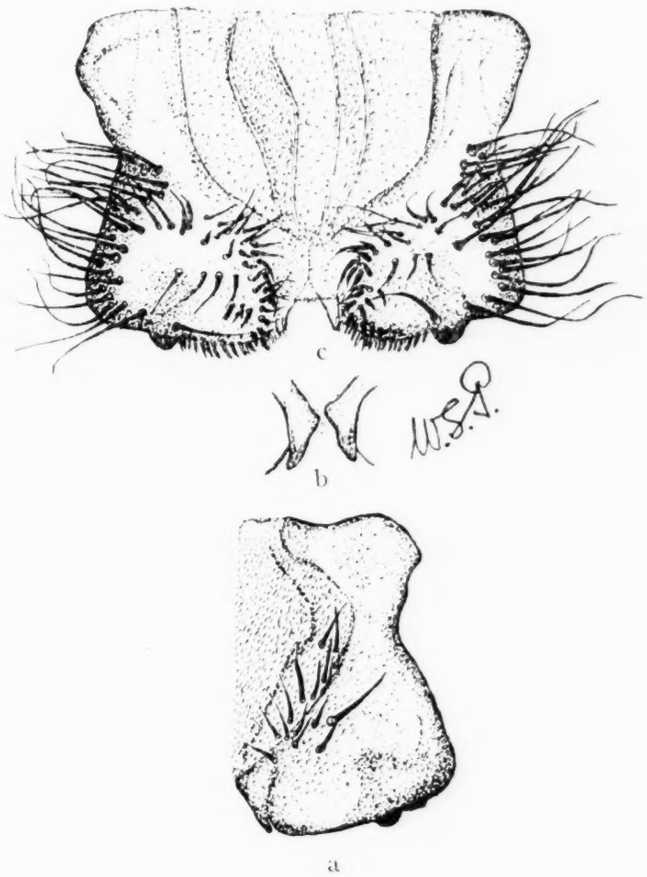


FIG. 22. *a*.—Dorsal view of one anal cercus of *swynnertoni* ; *b*.—Dorsal view of median lobes of anal cerci of *swynnertoni* ; *c*.—Ventral view of anal cerci of *swynnertoni*.

Glossina swynnertoni Austen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A small rather dark species, the tergal dark bands resembling those of *longipalpis* and *pallidipes* in that they are more extensive than those of *morsitans*. Antenna (fig. 21) very small, the arista

with rather long hairs. The thoracic and leg markings are very similar to those of *morsitans*. Abdomen with extensive dark-brown bands on a greyish or even ochraceous background. The median interruption rather narrow, its edges sharply defined and parallel ; the distal edges of the dark bands are parallel to the edge of the segments, and their inner margins are sharply truncate.

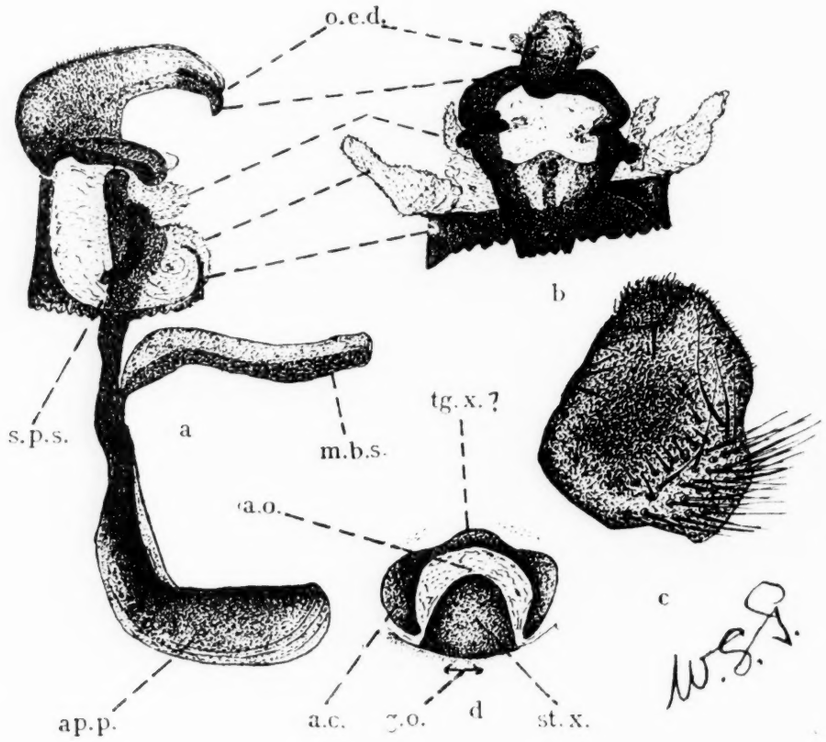


FIG. 23. *a*.—Phallosome of *swynnertoni* in side view dissected out of ninth tergo-sternum ; *o.e.d.*—opening ejaculatory duct ; other lettering as in fig. 3, *a* ; *b*.—Dorsal view of end of phallosome ; lettering as in fig. 3, *a* ; *c*.—Left paramere ; *d*.—Terminalia of ♀ *swynnertoni* ; lettering as in fig. 3, *b*.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci*. Fig. 22. Similar to those of *morsitans* but with a much more distinct tubercle on the distal border (nearer outer than inner margin) ; median lobes small and pointed and in dorsal view like the human foot turned outwards (fig. 22, *b*). *Phallosome*. Fig. 23, *a*, *b*. Very similar to that of *morsitans*. *Paramere*. Fig. 23, *c*. Except for size, very similar to that of *morsitans*. ♀. The female terminalia are illustrated in fig. 22, *d* ; there is nothing diagnostic in them.

DISTRIBUTION. HABITS. IMPORTANCE. *Glossina swynnertoni* is abundant in many parts of Tanganyika, where it is alone found. It mainly lives in acacia woodland. It is an important transmitter of pathogenic trypanosomes to man and probably to animals.

(*To be continued*)



Photograph of cases used for the transport of laboratory apparatus in Sierra Leone

H. R. Grubb, Ltd., Poplar Walk, Croydon

A CONVENIENT FORM OF CASE FOR THE TRANSPORT OF LABORATORY APPARATUS IN THE TROPICS

BY

R. M. GORDON

AND

T. H. DAVEY

(*From the Sir A. L. Jones Research Laboratory, Freetown, Sierra Leone*)

(*Received for publication* 8 *May*, 1936)

In many of the less civilized parts of the tropics the only form of transport available for laboratory stores is wooden boxes carried on the heads of native porters. It also frequently happens that the traveller in these regions is only able to spend a limited time at any one camp, and that the ' rest-houses ' which form his temporary quarters possess very little furniture on which he can arrange his apparatus. Under these circumstances, much time is wasted in unpacking and packing his boxes, and, this having been accomplished, further time is lost in trying to discover the particular article required amongst the stores which litter the floor.

The boxes shown in the accompanying photograph were designed in an attempt to reduce these difficulties to a minimum, and in practice they have proved very satisfactory, for, as can be seen from the illustration, once the lid has been let down the contents of the cases are exposed to view at a convenient height.

The boxes consist of wooden kerosine, or petrol, cases, strengthened by thin wooden battens, and provided with a hinged lid which when let down forms a convenient shelf. At the four bottom corners of the case are screwed metal sockets, measuring $2\frac{1}{2} \times 1$ inch. These sockets are similar in design to those provided on tropical camp-beds for the reception of the poles which support the mosquito-net, and can be made by any local blacksmith at a cost of a few pence. The supports for the boxes consist of wooden poles, measuring two feet long by an inch thick, and narrowed at one end to fit into the metal sockets just described ; owing to their length they are carried tied to the top of the box. After the case has been placed on its legs, greater stability is produced by separating the legs by means of the notched wooden laths shown in the photograph.

The contents of the boxes will, of course, vary with the needs of the individual worker, but in practice we have found it convenient to devote each box to a particular subject ; thus the box shown on the right contains all the apparatus necessary for the dissection of tsetse-flies and mosquitoes, while that on the left is divided into drawers and contains general laboratory stores.

We are indebted to Mr. D. J. Lewis, of the London School of Hygiene and Tropical Medicine, for taking the photograph which illustrates this note.

STUDIES IN RURAL HYGIENE IN THE TROPICS

BY

D. B. BLACKLOCK

(*From the Department of Tropical Hygiene, Liverpool School of Tropical Medicine*)

I.—THE DISPOSAL OF HUMAN EXCRETA

(*Received for publication* 13 *May*, 1936)

Many diseases which affect the inhabitants of rural areas in the tropics are due to the unsafe disposal of two excretions, the faeces and the urine. The list of diseases in which the infecting agent, bacterial, protozoal or helminthic, escapes to the exterior of the infected person's body by way of one or other of these excretions, is a long and impressive one : for the faeces it includes amoebic dysentery, ancylostomiasis, ascariasis, bacillary dysentery, balantidial dysentery, bilharziasis (*Bilharzia haematobia*, *B. japonica* and *B. mansoni*), cholera, clonorchiasis, dibothriasis, fascioliasis, fasciolopsiasis, heterophyiasis, the paratyphoid fevers, paragonimiasis, strongyloidosis, taeniasis (*Taenia solium* and *T. saginata*), trichuriasis, typhoid fever and undulant fever ; for the urine, bilharziasis (*Bilharzia haematobia*), spirochaetal jaundice, typhoid fever and undulant fever.

The complex modes of transmission of the pathogenic agents of many of these various diseases, and the often elaborate life-histories of the parasites and their vectors, have hitherto engaged a great proportion of the time of medical workers in the tropics. Partly as a result of the extensive knowledge obtained during this prolonged and difficult research, there is a somewhat natural tendency to be distracted by the very complexity of these life-histories from the one essential fact, which is that all these diseases can be practically eliminated by a single hygienic measure, namely, the safe disposal of the excreta.

Having had considerable personal experience in West Africa from 1921 onwards of the difficulties and expense attending the construction and maintenance of smoked-trench and other latrines for use in villages, the development of the bore-hole method of latrine construction in the Dutch East Indies, Malaya and India impressed me as holding out great possibilities for Africa.

In an address to the Royal Institute of Public Health in 1931, on the prevention of diseases in tropical countries, when referring to the bore-hole latrine and the trials of it then being made, I stated that, if it proved to be a sanitary success in African also, one could have no hesitation in saying that one of the most difficult problems of tropical sanitation there would have neared solution. The publications containing descriptions of this type of latrine were sent to Professor Gordon, who was in charge of our laboratory in Sierra Leone, in the hope and expectation that the Government authorities might instal some

of these latrines, preferably in the experimental village of Kaiyima ; but nothing appears to have been done. I believe it is correct to say that Sierra Leone has not, up to the present time, introduced any bore-hole latrines, although there are, I know, many parts of it quite suitable for them. The real danger in many villages in West Africa is not the future problematical infection of the water-supply by pathogenic bacteria, protozoa and helminths via the subsurface water, but the present actual infection of the surface water-supply in daily use by the direct disposal of gross excreta into it.

During a tour recently undertaken in Eastern countries I had an opportunity of enquiring and also of seeing to what extent this type of latrine was being installed in rural areas, and whether the people were accepting and using it ; a summary of these observations is given below.

In Assam, Dr. G. Fraser showed me at Labac the method of boring being used there. An auger 16 inches in diameter was employed, rotated by a length of two-inch pipe ; further lengths were screwed on as the hole deepened ; the auger and piping were suspended by a rope over the pulley in a block attached to four stout bamboos, about 30 feet high, tied together at the apex. The pipe was rotated by four labourers using a metal cross-bar of ingenious design, made locally, which gripped the pipe. Incidentally, this bore-hole latrine was being put down in the yard of a garden coolie at his own request.

At Digboi, Dr. A. S. Prowse showed me a series of houses occupied by clerks, at present supplied with a bucket-system of individual latrines situated six yards from the backs of the houses ; these bucket-latrines were to be converted into the bore-hole type.

At Cinnemara, Dr. D. Manson showed me a very complete system of bore-hole latrines for coolie lines ; these latter consisted of separate well-constructed dwelling-houses in straight lines. At the back, in the wide space which separated the rows of houses, every house had its own bore-hole latrine with a cheap, well-ventilated superstructure. The squatting-plate in use was of wood, but a new type is being made of concrete and is provided with a collar of concrete to extend two feet down into the bore-holes, which are 16 feet deep and 15 inches in diameter. The collar is designed still further to prevent fly-breeding, of which, however, there was at the time of my visit little evidence. The aperture in the temporary type of wooden foot-rest appeared unnecessarily large, and for small children possibly dangerous. A metal trough attached below it which carried the excreta to the back wall of the bore-hole did not appear to be an advantage, but it was explained that as a temporary expedient it was required owing to the large size of the aperture used.

At Mariani, Dr. George Macdonald reported that in his experience the coolies on the estates readily adopted the use of the bore-hole latrines, and that they were being increasingly installed.

In the Madras Presidency, Dr. Thirumaliat and Dr. R. M. Mathew took

me over the work at the Poonamalee Health Centre, and Dr. Mathew said that nowadays in some of the villages in this district he found no difficulty in getting villagers to use even public bore-hole latrines. This was the effect of education, and this again was the result of necessity, since the villages were frequently so closely built that there was not room for individual latrines. The people in the Madras Presidency have possibly advanced further in regard to this modern method because the bore-hole latrine was introduced here early and has become well known to the people, as shown in the report of the Public Health Commissioner with the Government of India (1932) on this subject. In one village inspected every house possessed a well of fair design, though lined with poorly jointed rings of clay. In this kind of place a bore-hole latrine might easily lead to serious pollution of the well-water; therefore, before bore-holes could be safely installed it would be necessary to ensure a supply of reliable water. If a proper piped or other safe supply can in future be obtained, many of the existing wells could be closed and probably adapted for latrines.

In some districts the Government of Madras has supplied the squatting-plates to villages at half the cost price, and has lent them the use of the auger, provided that the people themselves made the bore-holes. In one such area 6,000 latrines had been completed in this manner.

In Ceylon, at Negombo, three types of latrines are used: the bore-hole type in large numbers; where these are not possible, deep pit-latrines; or mound-latrines built around two metal tar barrels, with tops and bottoms removed, set one above the other: the lower barrel is sunk into a hole in the ground, and the upper occupies the mound. All these are provided with standard concrete squatting-plates (Pl. VI, fig. 3) and a well-ventilated and lighted superstructure.

Dr. S. F. Chellappah gave me details of the general regulations applied by him in the installation of bore-hole latrines; as they seem to cover most of the points which are important in popularizing this type and installing it safely, they are given:—

1. The latrine should not be more than 30 feet from the house.
2. It should not be nearer a well than 100 feet.
3. It should be kept away from fences or walls.
4. It should not be under a tree, which drips on to the roof.
5. The superstructure should fit closely round the squatting-plate and should be well ventilated and lighted.
6. The ablution-water should go into the bore-hole.

In Palestine I visited, with Dr. J. H. Pottinger, several villages in which latrines of the bore-hole type had been installed and in which they were in use. Dr. J. W. P. Harkness and Dr. V. L. Ferguson also took me to see the construction of the latrines in villages and the manufacture there on a large scale of concrete squatting-plates.

Several localities were found where the installation of this type of latrine

is attended with technical difficulties. For example, Dr. G. C. Ramsay and Dr. Burke demonstrated in Assam an area where the use of bore-hole latrines was prevented by a difficulty of strata. At the depth of a few feet a thick bed of large pebbles was encountered ; the auger was damaged by impact with these, and even when any progress was made the wall at once fell in ; this difficulty may later be overcome by some technical improvement as the result of experiment.

In the country near Rangoon, Colonel Cotter took me to Hlegu Health Unit Centre, and pointed out how they were endeavouring gradually to overcome certain very serious obstacles in Burma. The earlier bore-holes soon became blocked and filled up owing to the habit of the Burmans of using pieces of stick or bamboo with which to cleanse themselves, afterwards throwing these down the latrines. This is being successfully overcome by education and by the provision of tins to receive the sticks, which are burnt at regular intervals. Another difficulty is the proximity of the subsoil water to the surface of the ground during the rains. This is being overcome by building up an earth or brick structure for several feet above the ground level, in order to give depth enough to prevent fly-breeding. While the disposal of the faeces is said to go on quite satisfactorily in such sites, and the latrines do not tend to fill up, it is admitted that there appear to be at least two dangers in this method which require further investigation. The first is the fact that rats prove troublesome by burrowing into the mounds built up above ground-level. Wire netting is incorporated in the structure in an endeavour to prevent this, and fumigation by cyanogas is used to exterminate rats which have gained access to the structure. An equally important problem is that of flies and cockroaches. The question of fly- and cockroach-breeding in such short latrines requires careful investigation, as not enough is yet known on this subject, although it was stated here that there was no nuisance from fly-breeding. We noticed on raising the squatting-plate of one such old shallow bore-hole latrine that there were very numerous fly pupa cases on the soil beneath it. A still further difficulty is the provision of the necessary supporting casing for such built-up latrines ; here split bamboo was used for the purpose. All these points are being investigated here and the results will be of great interest, as this area appears at first sight an unpromising one for the making and functioning of bore-hole latrines.

A good adaptation of the bore-hole for the purpose of a urinal is used in Ceylon at Mirigama : the bore-hole is 25 feet deep ; there is a circular opening in the concrete slab, and two vertical plates of iron are set at right angles to each other over the centre of the aperture, thus giving four urinal compartments ; the whole is surrounded by a screen of wattle ; this type of bore-hole urinal has been found very useful in dealing with camps, fairs and pilgrimages, and causes no nuisance from flies or odour.

Dr. Stirling in Assam informed me that he had been using bore-holes as a means of providing villages with water, a useful temporary measure ; the people drew the water from the bore-hole well in a small bucket attached to a rope.

I went into some detail with regard to the various types of latrine in use, and especially with regard to the bore-hole latrine, feeling convinced that it was highly important to ascertain in each country what the local experience of it had been.

China stands in a category by itself in so far as the disposal of human excreta is concerned, owing to the utilization of human excreta in agriculture ; the bore-hole latrine in its present form seems to have no chance of acceptance there.

In all other countries visited, however, there is very abundant evidence that this type is being well received by villagers and that it is proving highly successful in use in suitable areas. Not only so, but even in areas which at first sight appeared to be somewhat unsuitable for it, as, for example, in the Burma Rural Area where several factors already mentioned, such as the habits of the people and the height of the subsoil water in the wet season, militate against it, the difficulties are being gradually overcome.

It was noted that in Malaya, Burma, India, Ceylon and Palestine, its use is making rapid headway, and it may be concluded that it is supplying a much-felt want and, at the same time, a perfectly good and simple solution of the disposal of human excreta in many areas.

At Mirigama in Ceylon there is a very remarkable residential institution for training rural school-teachers in several subjects, of which rural hygiene is one. The evidence of these teachers was sought, and they assured me that in all parts of the Island the standard squatting-plate and the bore-hole latrine were accepted willingly by the people, who expressed their complete satisfaction with them; they had no complaints and there were definite signs of the popularity of this innovation.

The evidence obtained in India was, I thought, particularly important, since this is a place where caste prejudices and customs might raise quite unexpected difficulties. At Labac I took the opportunity of discussing the question of the use of bore-hole latrines with Dr. Ramsay and Dr. Fraser, and also with the Indian dispenser there, who has had thirty-six years' experience of all sorts and conditions of Indian labour. The dispenser expressed the opinion that bore-hole latrines met one of the great sanitary requirements of the Indian rural dweller of whatever rank, and especially of the coolie. He had found by observation that the various kinds of Indian labourer were only too willing to have them installed, and that they used them and maintained them in very good order. An essential condition was, however, that only one individual, or the members of one family, should be allowed to use any one latrine. He stated that all kinds of labourers, as soon as they had observed the simplicity and privacy of this kind of latrine, began to demand it.

Public latrines of the bore-hole type, however, were not yet popular, and no one would accept the responsibility of keeping them in good condition. Where these had to be installed, as in factories, it was invariably necessary to

provide a sweeper to look after them, and the sweeper himself required careful supervision.

One of the most important considerations is the fact that in favourable ground bore-hole latrines can be installed for each house at convenient and safe distances, and in such a way as to ensure that privacy which all people of whatever race desire in such matters.

A word may be said as to the degree of safety of disposal which the bore-hole latrine affords. There are several directions in which this type requires to be further investigated, since it would be an error to assume that it will be safe in all circumstances. The questions of mosquito- and fly-breeding, and the rat and cockroach nuisance must all receive attention, as well as the important one of choice of site. There is also much experimental field research to be done in other directions, in order to make this inexpensive and readily acceptable method of disposal as safe as possible.

As regards cost, the bore-hole latrine has some advantages over other types still in use. The bore-hole latrine shown in Pl. VI, fig. 4, cost less than 10 rupees, including the bore-hole, squatting-plate and superstructure; the more elaborate double bucket-latrine of concrete (Pl. VII, fig. 3) cost 40 rupees for each compartment; the disused portable metal latrine shown in Pl. VII, fig. 1, cost 275 rupees. Only the mound-latrine (Pl. VII, fig. 4) compares with the bore-hole in cheapness, and this is intended for special sites.

In India, the question of supplying the apparatus and the information necessary for making the bore-hole latrines is attracting the attention of manufacturers. For example, in Calcutta, the firm of Ivan Jones, Ltd., issues descriptive pamphlets and blue prints of a ' type plan of the bore-hole latrine,' with all measurements, and they have already considerably reduced the price of the augers.

I have enumerated over a score of diseases, each of which could be eliminated if every member of the community were educated and trained to dispose of their faeces and urine safely. It has also been shown that for rural areas the most simple, inexpensive and safe method appears to be the use of the bore-hole latrine. Further, it has been shown that in all countries visited, with the exception of China, the evidence obtained is that this type will be accepted readily by the people in rural areas.

REFERENCE

Annual report of the Public Health Commissioner with the Government of India (1932). vol. I.

PLATE VI

EXPLANATION OF PLATE VI

Fig. 1. View of boring apparatus and supporting bamboos; note hinged lid (marked by arrow), with an aperture to guide the pipe used.

Fig. 2. The guide hinged lid open and the auger full of earth withdrawn.

Fig. 3. Concrete squatting-plates reinforced with $\frac{1}{4}$-inch metal rods.

Fig. 4. Bore-hole latrine (marked by arrow) adjacent to tuberculosis sanatorium.



FIG. 1



FIG. 2

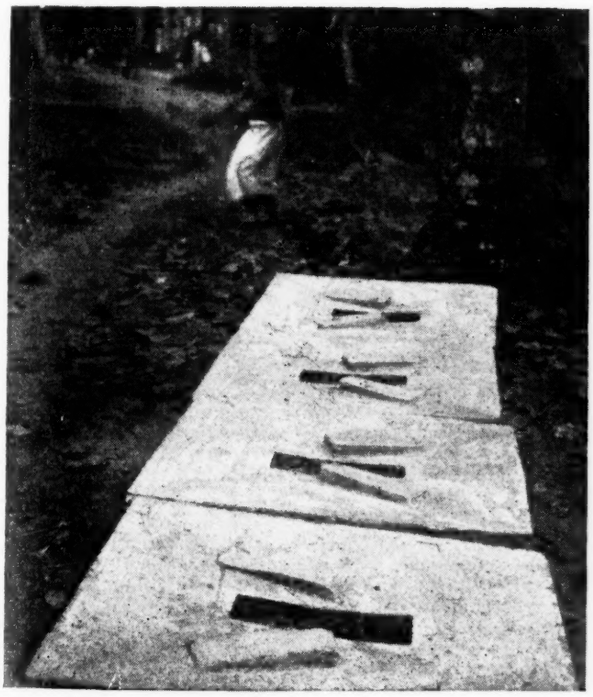


FIG. 3



FIG. 4

H. R. Grubb, Poplar Walk, Croydon

EXPLANATION OF PLATE VII

Fig. 1. Bore-hole latrine now in use (marked by arrow); beside it a portable latrine of metal (cost Rs. 275), now disused.

Fig. 2. Close-up view of the interior, showing squatting-plate (marked by arrow), bamboo poles and matting sides.

Fig. 3. Concrete bucket-latrine.

Fig. 4. Mound-latrine built up round a metal drum.



FIG. 1

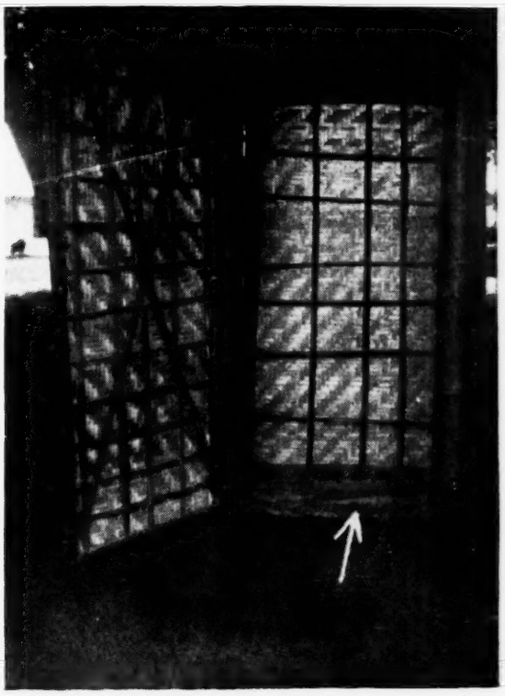


FIG. 2

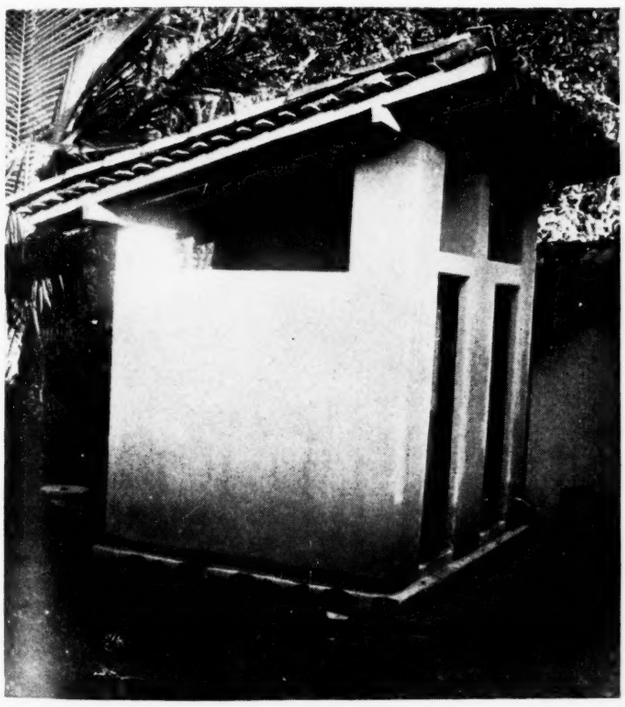


FIG. 3



FIG. 4

H. R. Grubb, Ltd., Poplar Walk, Croydon.

STUDIES IN RURAL HYGIENE IN THE TROPICS

BY

D. B. BLACKLOCK

(*From the Department of Tropical Hygiene, Liverpool School of Tropical Medicine*)

II.—THE CULTIVATION OF DENSE SHADE PLANTS AS AN ANTI-MALARIA MEASURE

(*Received for publication* 19 *May*, 1936)

The methods of surface drainage and the sub-surface method introduced by Watson in Malaya, which are carried out successfully there and in many parts of the world on a large scale, have given very disappointing results in the Dooars and in Assam. Various reasons are adduced to account for what is regarded in many places and by many people there as a definite failure. The importance of the experimental work which has been carried on for some years in Assam as a direct effect of the poor results of drainage, renders it all the more necessary to state some of the reasons generally given to account for its lack of success. During a recent visit to India, I was fortunate in the fact that it was possible for Dr. G. C. Ramsay, Director of the Ross Institute of Hygiene in Calcutta, to conduct me through parts of the Dooars of Northern Bengal and of Assam to see the conditions on many of the tea-gardens. The medical men in Assam with whom I discussed the drainage methods for the control of malaria were almost unanimous in stating that the methods had been given a reasonable chance but had not answered expectations. Among the reasons assigned was the heavy rain in Assam compared with that of Malaya ; also the fact that this heavy rain falls during a limited number of months in Assam, whereas in Malaya the smaller rainfall is spread over the year much more evenly. Dr. B. Barrowman in Klang informed me that in going over the records of the past twenty years for Malaya he found that each month of the year had at one time or another shown the highest rainfall.

The next point emphasized was that the ground was often so flat that the necessary fall for good local drainage could not be obtained ; another cause to which failure was attributed was the multiplicity of the breeding places which had to be treated.

The results of the existence of the first three of these factors were that a contour drain dug and left open was soon washed out by the heavy rains and rendered not only useless but dangerous. On the other hand, if it were converted into a sub-surface drain it completely failed to deal with the great volume of water. Central drains, if unconcreted, suffered the same fate as did the rough open contour drains, and even if concreted they still failed to get rid of the immense amount of water in the rains. The end results were an increase

instead of a diminution of anopheline breeding water, owing to the fact that partly efficient drains revealed new and dangerous seepages, while inefficient drains overflowed and formed additional breeding places.

In connection with these adverse statements, however, I obtained from Mr. Casebourne, the superintendent of the Doomdooma group of gardens, some evidence which appeared to have an important bearing on the fundamental causes of the non-success of the drainage. Over a period of some years the engineers in charge of the anti-malaria work had done a great deal of surface and sub-surface drainage. While the final outcome from the anti-malaria aspect was far from being a success, yet there was a marked lowering of the surface-water level over a very large area. Mr. Casebourne put the lowering at two feet or more, pointing out to me areas which could now be shot over, but which before drainage had been impassable swamps. Such lands had, as a result of the drainage, been greatly used in recent years by the coolies for rice cultivation. In the years since the drainage was commenced, some 500 acres had been added to the cultivable rice-land of the gardens. The fall of the water, however, was not by any means sufficient to get rid of the innumerable seepages in which *Anopheles minimus* bred ; in fact, it increased their number.

This evidence was especially interesting as showing that the idea of drainage in all probability was sound enough, but that some unexpected circumstance had escaped attention. It seemed to emerge that the basic factor which rendered the drainage methods a failure was the lack of adequate fall in the natural streams to which the local drains were led, and, further, in the main rivers into which these feeder streams entered. It was in fact possible, by standing on a bridge over a stream of considerable width, which should have acted as an easy run-away for the water from a large portion of the land drainage system at Doomdooma, to note that the water in this tributary of the main river was practically stagnant. It appeared quite obvious that the stream as it was could not give a sufficiently rapid run-off to the additional water which a heavy rain might suddenly put into it via the system of land drains. If the drains had not existed, a steady soddening of the ground over the whole area during the rains would have taken place, and after the rains eased off there would have been a gradual filtering away into the streams. Such a sodden marshy condition was doubtless safer than the state of affairs which supervened when the clearing of the jungle was done and the drains put down ; after that the bulk of the surface water of the estates could run away quickly, but, owing to the lack of adequate fall in the streams, the drains could not be put deep enough to eliminate seepages, but only revealed more of them, so that conditions were worse. It may well be the case, then—and it appears probable that it is so—that the failure of drainage methods in certain parts of Assam is not to be attributed to any purely local obstacles to this well-tried system, such as the heavy rains or the local lack of slope in individual gardens, or even the multiplicity of breeding places, but to a much more fundamental drawback, easily

unforeseen and not local, namely, a lack of fall in the tributaries of the large rivers and in the large rivers themselves.

When it became obvious that in certain regions the drainage method was ineffective, and that in some others it was even introducing fresh dangers, means were sought to replace it by other methods. Hence arose the idea of shading streams by dense artificial shade, a conception based on knowledge of the bionomics of the chief vector, *Anopheles minimus*. Dr. Ramsay has been among the foremost exponents of this system in practice, and it is a method which has the advantage, where conditions are suitable, of being cheap. Since the method is one which, for reasons stated later, may prove of great importance in other parts of the world, it seems opportune to draw attention to its progress in Assam by reference to personal observations made on the spot.

At Doomdooma, Dr. H. Flack showed me areas where extensive trials of drainage had failed to give good results. There is no stone in this and many other tea-gardens in Assam, so that the making of stone drains is not feasible ; the alluvial soil, on the other hand, is not suitable for open drains, as the soft earth falls in readily, and the drains are easily scoured out by the flood-water and badly damaged. Dr. Flack at the same time showed me the work being done in the way of suppressing malaria by planting shade plants over *Anopheles minimus* breeding streams and also in marshy areas. Basak, a plant with large oval leaves, grows quickly and provides good shade. Gradual control of malaria is being attained by the encouragement and augmentation of such naturally growing shade plants, and also by extensive planting on difficult breeding streams.

Dr. M. E. T. Burke at Tinglas demonstrated several methods employed for the control of man-made malaria due to the irrigation. One of them was this modern method of planting dense shade plants close along the edge of the water channels ; Lantana and Eupatorium were the chief plants used.

On the Ross Estate the water for the factory is led along through the tea-gardens in a very long channel, in which the water is entirely concealed by a thick high hedge of Lantana planted three years ago.

Dr. A. M. Warwick took me over his gardens where the drainage methods had been tried and failed, owing, it was stated, to the heavy rains. The method now used for streams was dense shading with Tarapat, Lantana and Eupatorium.

On the Cinnemara Estate, Dr. D. Manson showed me the great extent to which the dense shading of *Anopheles minimus* breeding streams had been carried ; miles of well-covered streams were seen. Hibiscus is good and grows rapidly, but cattle eat it, so that it has to be protected from their depredations. Eupatorium has also proved of great value, but care is required in its use ; since its ripe seeds spray out to some distance around, seeds must be cut off before they are ripe, if they are situated near the tea ; otherwise they take root among the tea bushes and become a weed pest. Dr. Manson has discovered that the berries

of Durantha, a spiny plant with a blue flower which has been much used for dense shade over streams, produce a narcotine which is lethal to anopheline larvae; this poison may have some effect in reducing larvae in small streams if the berries which fall into the water are numerous. Cattle and goats do not eat this spiny plant, which grows well in several areas. Pl. VIII, fig. 3, shows a hedge of Durantha over a man-made *minimus* breeding place in Dooars; the hedge is three years old, and the shade is so dense that there is no grass or weed under it; the cost is £2 10s. per mile.

Dr. G. Fraser at Labac gave me the opportunity of seeing many hollows and marshy areas which had formerly been central-surface and contour sub-surface drained, with disastrous effects as regards increase of *Anopheles minimus* breeding, and over which dense shading was now being practised. One stream of about 8 feet wide, which could not be covered satisfactorily by the shade of the lateral growth of the usual plants employed, was being planted with bamboo to produce the required shade.

The quality and quantity of shade required. Many records exist in the literature of anopheline breeding places in deep, dense shade or in heavy shade. But the so-called heavy shade of natural jungle or bush, which appears to be what is meant in this description, seems to be a very different thing, in its final effect, from properly planted artificial shade. An important distinction is made by Dr. Ramsay in regard to the terms bush, jungle, heavy shade or jungle shade as found in nature, and the ' dense shade ' obtained as a result of artificial planting and growth. He found that casual or natural jungle cover is not, as a rule, sufficiently dense to prevent *A. minimus* breeding in numbers in portions of the water under it. In order effectively to prevent this species breeding, the shade plants must be so close together that they exclude the sun from the water, with the result that no green vegetation grows in it. Apart from anything else, it is thought that the larvae of *A. minimus* require grass or weed in the water to which they can cling with their tail hooks, and in the absence of this they will be washed away.

In Freetown, Sierra Leone, Blacklock and Evans (1926) described how *Anopheles gambiae*, which was present in various parts of a relatively lightly shaded stream passing through the town, was not found in the upper part of the same stream where it descended the rocky well-shaded hill called Mount Aureole, and how in this stretch *A. smithii* replaced it. It was not clear whether the different distribution of these two species was dependent on the amount of sunlight reaching the water or on other factors as well. Now the natural shade over this stream on Mount Aureole was not to be compared in its regularity and density with the artificially planted shade which I saw being grown over streams in Assam.

This opens up a very important line of experiment for all other species of anophelines besides *A. minimus*. It may be found that the planting of really dense shade will eliminate the breeding of various other species of anophelines

in many different regions in Africa and elsewhere. For example, will even *umbrosus* and *smithii* survive in really densely planted shade ? In this connection the following observation appears to be relevant. At the quarantine station on Pulau Jerejerak near Penang, Dr. S. W. Eveson showed me a method which was used there for wells in order to prevent the breeding in them of *A. kochi*, *vagus*, *barbirostris* and *sinensis*. A small building of wattle and daub with a thick thatch (atap) roof was built over the well, and a door of overlapping sacking was provided ; the darkness was said to be quite deterrent to anopheline breeding.

With reference to the rate of growth of shade plants in certain areas, at Rangapara Mr. Forbes showed me in his garden a hedge of a species of Hibiscus, which he had planted just over a year ago. The stems were in some cases $1\frac{1}{2}$ inches thick, and the hedge was 8 feet high.

It is worth considering that in the case of a stream properly planted with dense shade the adult female anopheline has to face a large number of risks of injury if she is to penetrate successfully through down to the water. She may be injured by leaves, branches, predatory insects, spiders and spiders' webs ; after oviposition she has again to traverse a very difficult maze to get out safely. If the larvae are not destined to survive in water deprived of green vegetation and sunlight, she will be unlikely to repeat her hazardous journeys, even if she is successful in negotiating the dangers safely on the first occasion.

In view of the success which appears to be attending the efforts at controlling *Anopheles minimus* breeding by the use of ' dense shade,' it seems well worth while to give this method a thorough experimental trial in suitable places where other species of anophelines constitute a problem. In each locality specific tests will be required, using the various local shade plants which appear to thrive in the immediate vicinity of water and in marshy areas.

REFERENCE

BLACKLOCK, D. B., and EVANS, A. M. (1926). Breeding places of anopheline mosquitos in and around Freetown, Sierra Leone. *Ann. Trop. Med. & Parasitol.*, **20**, 59.

EXPLANATION OF PLATE VIII

Fig. 1. An *A. minimus* stream to be shaded.

Fig. 2. Shrubs planted for shade beginning to grow.

Fig. 3. Good growth of a shade plant.



Fig. 1



Fig. 2



Fig. 3

H. R. Grubb, Ltd., Poplar Walk, Croydon.

THE BRITISH SPECIES OF THE SUBFAMILY SARCOPHAGINAE, WITH ILLUSTRATIONS OF THE MALE AND FEMALE TERMINALIA

(*Continued from vol.* 29, *page* 532)

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Sarcophaga haemorrhoa Meigen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂. This is one of the ' red-tailed ' species having a bright-red tenth tergum (the so-called second genital segment). It is easily separated from *striata* F. by its outward-curving series of frontal setae, which are shorter and quite straight in *striata*; and by possessing 3 D.C., hind marginals on third (second visible) abdominal tergum, and a row of setae at the base of both the first and third longitudinal wing veins, and is distinguished at once from any other allied British species excepting some individuals of *frenata* Pand. It is very like *frenata* in all external characters (see previous paper) ; but the two species can, we think, be separated by the differences in the setae of the wing veins, in spite of their variability ; they are usually few and long in *frenata* and more numerous, shorter and closer together in *haemorrhoa*. On the first vein *frenata* usually bears two or three long, rather widely separated bristles ; *haemorrhoa* a close series of perhaps ten or twelve short bristles extending about half way from the fork to the end ; *frenata* is especially variable in this character—the first vein may be entirely bare, or there may be an extended series ; *haemorrhoa* is apparently fairly constant, but Böttcher mentions a French specimen seen by him in which the first vein was bare. The frons is narrower in *haemorrhoa* than in *frenata*, but this again is a variable character; Lundbeck says that that of *haemorrhoa* is only one-third of eye-width; we find it to be wider than that, and even fully half eye-width. The first genital segment (tergum 7) in *haemorrhoa* is about the same size as the second (tergum 10), no longer and no wider and bearing white tomentum ; in *frenata* tergum 7 is altogether larger, longer and wider, and usually entirely shining. The leg chaetotaxy differs somewhat ; in particular there is a full antero-ventral series of long, strong setae on the hind femora in *frenata*, whilst in *haemorrhoa* there are only a few long irregular bristle-hairs in that position. A useful character for distinguishing this species may prove to be the length of the third costal section, which is even shorter than the fifth ; in *frenata* it is decidedly longer.

Frons about half eye-width or less ; third antennal joint barely half as long again as the second ; genal setae well developed and numerous, forming a fairly

regular row ; no presutural acrostichals, but numerous bristly hairs in this region, some rather long and strong ; scutellum with a pair of crossed apicals, long, strong preapicals and strong basals, but only weak discals ; tergum 7 with row of strong marginal setae ; costal thorn variable, generally small ; third and fourth sternites with only short pubescence.

LEG CHAETOTAXY. Front femora as usual, no antero-ventral series; postero-ventral row long and complete, basal setae thinner and hair-like, becoming thicker towards apex. Mid femora with few short, strong, irregular setae in basal half of antero-ventral series, and a regular row of fine hairs in apical half ; on postero-ventral aspect long bristle-hairs, becoming shorter towards apex, where there is only a short row of fine comb-like hairs ; hind femora on antero-ventral aspect, with an irregular series of mixed thin and stronger bristle-hairs, and on postero-ventral aspect a more regular series of long thin bristle hairs ; hind tibiae usually more or less fringed, but this is a very variable character, the fringe being chiefly in evidence in the case of the larger and more robust individuals.

♀. The female resembles the male in most characters, and runs down with it in the keys, having also red terminal segments. It again has to be compared with the ♀ *frenata*, which it resembles very closely. The character referred to, in the notes on *frenata*, of the structure of tergum 6 seems to hold good and to enable the two species to be separated with ease. In *haemorrhoa* this tergum is complete and is not divided into two separate plates, whilst in *frenata* it is seen to be deeply indented dorsally and on dissection proves to consist of two distinct plates. The short third costal section will also probably prove a useful distinguishing character.

Frons fully of eye width ; no apical scutellars, but the discal pair stronger than in the ♂. LEG CHAETOTAXY. Front femora with complete postero-ventral series of long strong bristles ; mid femora on antero-ventral aspect with two strong bristles in basal portion, and a regular row of fine hairs in apical half ; on postero-ventral aspect with four or five longer, thinner bristles in basal portion; and an even less comb-like series of fine hairs towards apex ; hind femora with irregular scattered series of longish thick bristles on both antero and postero-ventral sides.

NOTES. *S. haemorrhoa* is not one of our abundant species, but is not uncommon ; we have it from a number of localities, east, west and south, but not at present from Scotland or Ireland. According to Böttcher it occurs over nearly all Europe, north and south, and is usually common.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. The ♂ terminalia of *haemorrhoa* are illustrated in fig. 1 ; they should be compared with those of *frenata* illustrated in our second paper. Sternum 5 (fig. 1, *b*) is strikingly like that of *frenata*, the differences being slight. The anal cerci (fig. 1, *c*, *d*) are longer than those of *frenata*, and have a very characteristic appearance in side view (fig. 1, *d*), suggesting the head of a bird with a long stout beak. The phallo-

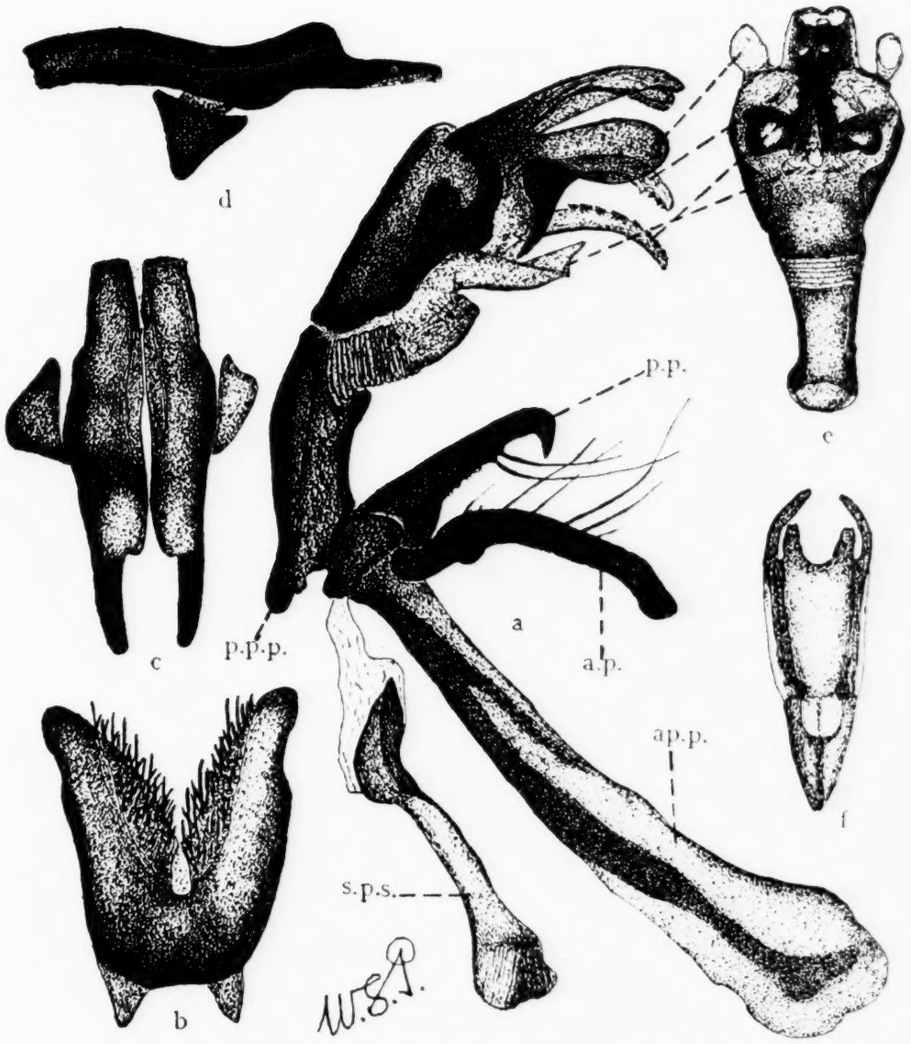


FIG. 1. *a*.—Phallosome and one paramere of *haemorrhoa* in side view ; *a.p.*—Anterior part of paramere ; *ap.p.*—Apodeme of phallosome ; *p.p.*—Posterior part of paramere ; *p.p.p.*—Posterior process of phallosome ; *s.p.s.*—Sperm pump sclerite ; *b.*—Fifth sternum ; *c.*—Ventral view of anal cerci and distal segments of ninth coxites ; *d.*—Lateral view of anal cercus and distal segment of ninth coxite ; *e.*—Dorsal view of end of phallosome ; *f.*—Ninth tergo-sternum.

some is similar to that of *frenata* ; it is long but has several more and longer processes at the end on the dorsal side, as shown in fig. 1, *a* ; the end is also much wider than that of the phallosome of *frenata* (fig. 1, *e*). The two parts of the paramere too are strikingly like those of the paramere of *frenata*.

♀. The ♀ terminalia of *haemorrhoa* are illustrated in fig. 2. Tergum 6, unlike that of the ♀ *frenata*, consists of a single plate (fig. 2, *e*), affording a certain means of distinguishing the two females, the terminalia of which are otherwise somewhat similar. Sternum 7 has a distinct plate attached to its distal end, which appears to be sternum 9, and it lies at a lower level, as is shown in side view in fig. 2, *d*. Sternum 10 has a rather square plate attached to its lower part. The signum is wanting.

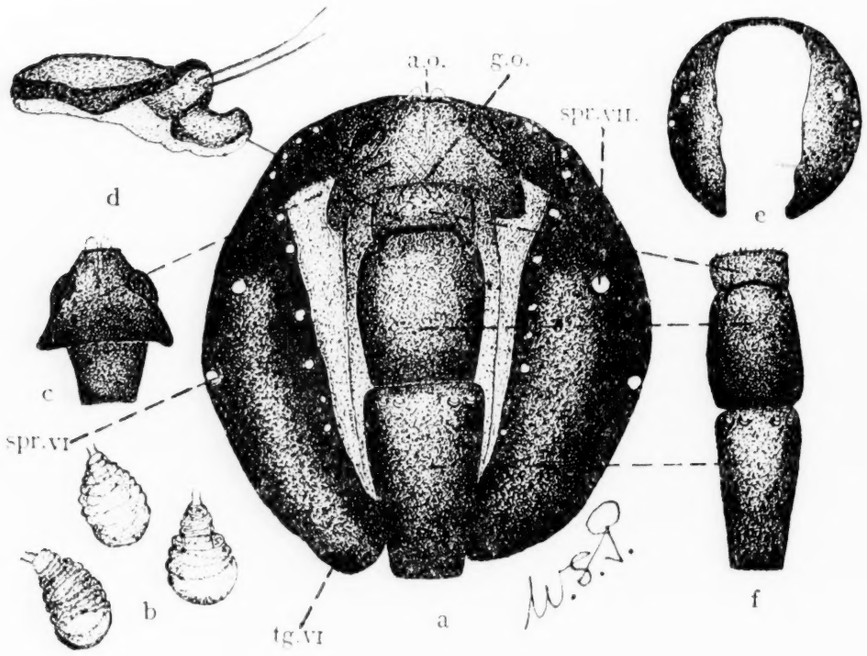


FIG. 2. *a.*—Ventral view of terminalia of ♀ *haemorrhoa* to show diagnostic characters ; *a.o.*—Anal opening ; *g.o.*—Genital opening ; *spr.vi*, *spr.vii.*—Sixth, seventh spiracle ; *tg.vi.*—Sixth tergum ; *b.*—Spermathecae ; *c.*—Tenth sternum and anal cerci ; *d.*—Seventh, ninth (?) sterna in side view to show how the latter is depressed below the end of the former ; *e.*—Sixth tergum showing that it consists of a single plate ; *f.*—Sixth, seventh and ninth (?) sterna.

Sarcophaga melanura Meigen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂. *Melanura* is one of the species of our British *Sarcophaga* which it is easy to recognize. It bears a general resemblance to those of the *carnaria* L. group, varying in size but usually about as big as an average *carnaria* ; but in the keys it runs away from them through the possession normally of 3 D.C. only (Böttcher says that there is sometimes a small fourth one in front). Then, having no marginals on the third (second visible) abdominal tergum, it falls to be compared amongst the British species only with *incisilobata* Pand. and *hirticrus* Pand., two distinct species separable at once by their much narrower frons, *melanura* being notable for its unusually wide frons, which approaches eye-width even in the ♂ (it is at least four-fifths of eye-width). This wide head, with the frontalia twice the width of an orbit, and the orbits, genae, etc., brownish in colouring, is characteristic of the species and leads to easy recognition.

The antennae are not long, third joint about half as long again as second ; arista with long ciliation ; genae with about a dozen fine irregularly placed setae ; strong verticals, inner and outer. Thorax with three strongly developed longitudinal lines ; no presutural acrs., but densely hairy between the presutural D.C. ; three postsutural D.C., with two or three presutural as well ; scutellum with a pair of crossed apicals, long strong preapicals and strong basals, but no other marginals, one pair of discals. Abdomen with a pair of marginals on fourth

tergum, and a full series on fifth ; a row of short thick setae on hind margin of first genital segment (tergum 7) ; both genital segments (terga 7 and 10) devoid of tomentum and shining ; sternites with longish hairs on basal segments, but shorter on third and quite short on fourth. Wings with small costal thorn ; setae on third longitudinal vein reaching nearly to discal cross vein.

LEG CHAETOTAXY. Front femora with no antero-ventral series, but postero-ventral series complete, with much dense pubescence on basal half ; mid femora with antero-ventral series nearly complete, short, strong and regular, but forming no ' comb ' at apex ; postero-ventral series with basal half more densely hairy, and on apical half forming a fairly definite strong comb ; hind femora with irregular incomplete series of medium length setae on antero-ventral aspect, and a complete series of long bristle-hairs forming a fringe on postero-ventral aspect ; hind tibiae with dense long fringes on antero- and postero-ventral aspects ; mid tibiae with none.

♀. As in the case of the ♂, so in that of the ♀, this is one of the species which it is comparatively easy to recognize. It possesses the same general characters in the ♀ as in the ♂, running down in the keys to the same point, and its very broad frons enables it to be identified in the ♀ as well as in the ♂ sex. The ♀ frons is nearly half as wide again as an eye, whilst in *hirticrus* Pand. and *incisilobata* Pand., against which it comes in the keys, it is only about eye-width, and the general brown colour of the tomentum of the head assists identification. The description of the ♂ applies in general to the ♀ ; it possesses no scutellar apicals, these usually failing in the ♀♀ in this genus. The external view of the terminalia is very characteristic ; these show dorsally a wide deep rounded groove. Dissection (see below) shows tergum 6 to be divided dorsally into two plates, and below these are unusually developed and project considerably on each side, and the groove is the hollow lying in between these plates. One effect is that it appears as if the terminal opening were on the dorsal aspect of the insect instead of on the ventral.

LEG CHAETOTAXY. Fore femora as in the ♂, with no antero-ventral series ; with long and strong postero-ventral series, but without any of the dense pubescence found in this place in the ♂ ; mid femora on antero-ventral aspect with about three short thick setae on basal portion, and then, from middle on, a regular comb of fine short hair-like setae ; on postero-ventral aspect about four long scattered strong setae on basal half, and then also a fine comb to apex ; hind femora as in the ♂, with a fairly complete but irregular antero-ventral series, but with no postero-ventral fringe such as the ♂ has, only a few long hairs basally.

NOTES. This is one of our less abundant species, but it is not rare, and where it occurs it is usually common. We have found it to be especially a coastal species, and have it from such places as Studland (Little Sea area), Hengistbury Head, St. Ives, Torquay, Yarmouth, etc. ; we have, however, also taken it in

a Warwickshire wood. We have one specimen from Glengariff, Ireland (H. W. Andrews), but none from Scotland. Böttcher says that it occurs throughout the Palaearctic region, and is generally common.

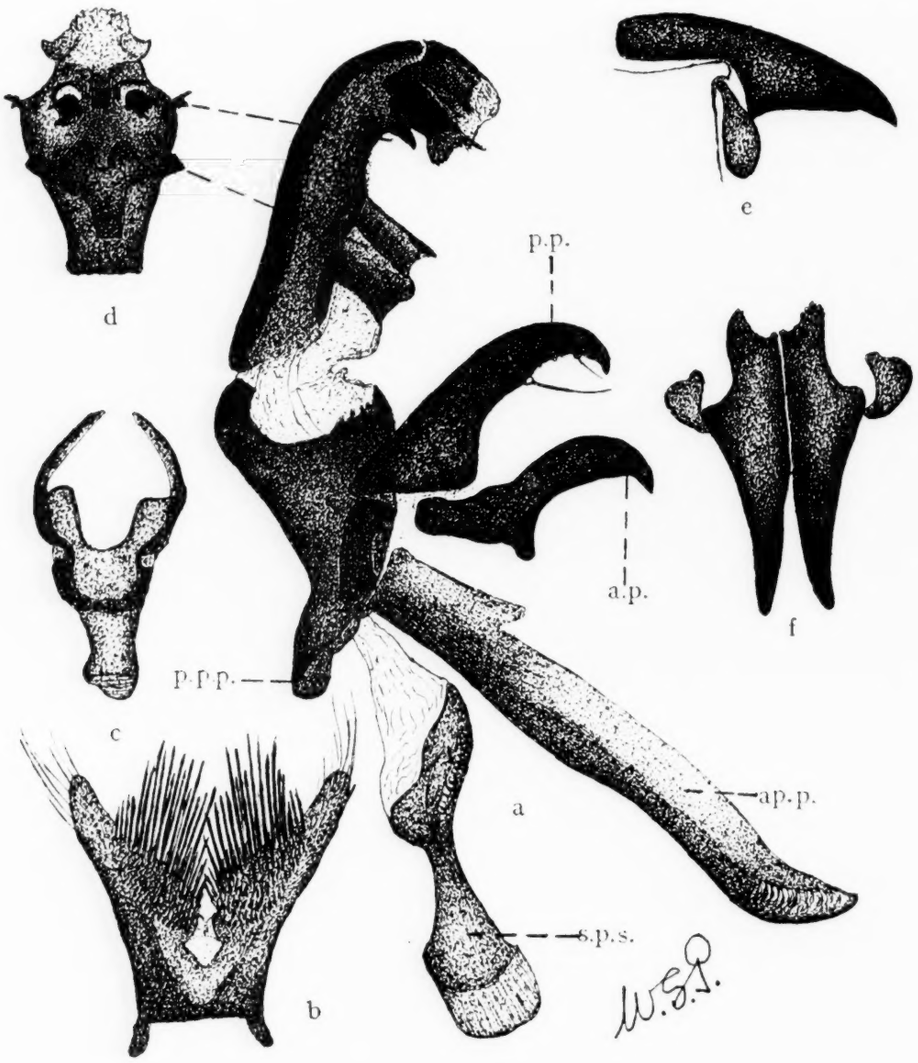


FIG. 3. *a*.—Phallosome and one paramere of *melanura* in side view ; lettering as in fig. 1, *a* ; *b*.—Fifth sternum ; *c*.—Ninth tergo-sternum ; *d*.—Dorsal view of end of phallosome ; *e*.—Lateral view of anal cercus and distal segment of ninth coxite ; *f*.—Ventral view of anal cercus and distal segment of ninth coxites.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. The ♂ terminalia of *melanura* are illustrated in fig. 3. Sternum 5 (fig. 3, *b*) is similar to that of *scoparia* (fig. 5, *b*), but has much stouter, stiffer hairs on the inner sides. The anal cercus as seen in profile (fig. 5, *e*) is a simple, rather short plate, ending in a point ; the anal cerci in ventral view are shown in fig. 3, *f*. The phallosome is long and simple, and has only short dorsal processes at both ends of the distal part ; these are well shown in the profile view in fig. 3, *a* ; the dorsal view of the end of the phallosome is shown in fig. 3, *d*.

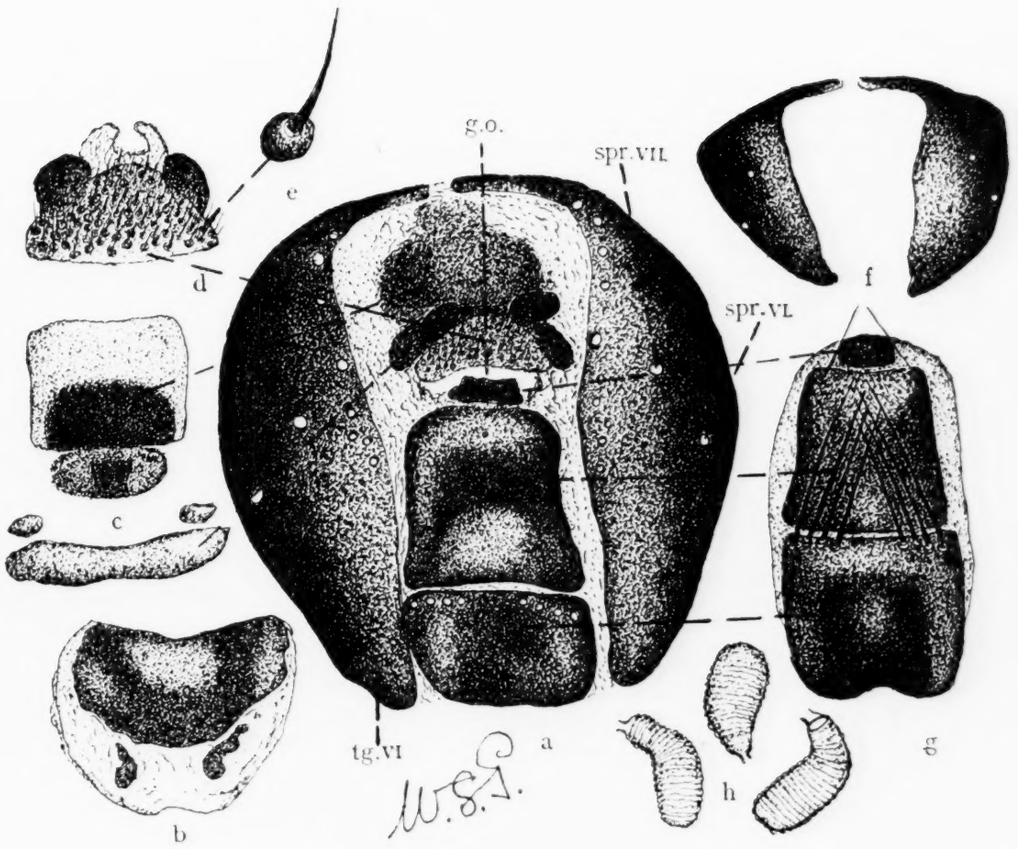


FIG. 4. *a*.—Ventral view of terminalia of ♀ *melanura* to show diagnostic characters ; lettering as in fig. 2, *a* ; *b*.—Signum ; *c*.—Plates (7th, 9th terga ?) postero-dorsal to anal cerci ; these are very characteristic of this species ; *d*.—Tenth sternum and anal cerci ; *e*.—Bristle from tenth sternum ; *f*.—Sixth tergum showing its division into two parts ; *g*.—Sixth, seventh, ninth (?) sterna ; *h*.—Spermathecae.

♀. The ♀ terminalia of *melanura* are illustrated in fig. 4. Tergum 6 consists of two plates, and the space postero-dorsal to the anal cerci is occupied by three plates, which are illustrated in fig. 4, *c* ; it is possible that these represent terga 7 and 9. They afford a good diagnostic character. Sternum 7 has a small plate at its distal end, which may be sternum 9. The signum is illustrated in fig. 6, *b*.

Sarcophaga scoparia Pandellé. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂. This is perhaps our largest and finest species of *Sarcophaga*, some of the biggest specimens measuring 16 mm. in length over all, although, as usual, size varies and smaller individuals are frequent. It is a darker-looking insect than *carnaria* L., with which comparison would naturally be made, and, as it possesses much long shaggy pubescence, is more striking and perhaps handsomer. It runs down in the keys, through entirely black terminalia, four well-developed D.C., no presutural acrs., and bare first longitudinal vein, to the same group of species as *aratrix* Pand. (see below), but can be distinguished from this and other allied species by the

long dense pubescence on all the sternites, together with the unusual development of the long fringes of hairs on all the legs, especially on the hind tibiae. *Aratrix* Pand. is easily separated from it by the tooth-like projection at the base of its apical sternite ; and *carnaria* L. and *vicina* Vill. have marginal setae on the second abdominal segment which are wanting in *scoparia.* The other allied British species, *albiceps* Mg., *similis* (Meade) Pand. and *tuberosa* Pand., have only very short hairs on at least the third and fourth sternites, and, although *teretirostris* Pand. has rather longer hairs on all its sternites, yet they are not nearly so long and strong as in *scoparia*, the fringes on the legs and general pubescence are much less developed, and it is a smaller insect. *Scoparia* is, in fact, one of those species which will probably be recognized almost at sight.

Frons about two-thirds eye-width at narrowest part ; no outer vertical bristles ; genae with about a dozen irregularly placed and variable setae ; third antennal joint twice as long as second ; arista with long ciliation ; scutellum with a pair of apical crossed and recurved bristles, a pair of small discals near to them, and usually three marginals on each side. Fourth tergite with a strong row of marginals ; seventh tergite (first genital segment) with a marginal row of setae, and with silvery tomentum on posterior portion ; fifth sternite with no brush, but with row of marginal setae. Wing with third costal section long, much longer than fifth ; no costal thorn. Leg Chaetotaxy. Front femora with much long dense pubescence, out of which a postero-ventral row of strong bristles develops apically ; no long pubescence on front tibiae ; middle femora also with much long pubescence, and on antero-ventral aspect a complete series of short, thick, thorn-like setae, on postero-ventral aspect a short similar series of rather longer setae on apical third only ; middle tibiae have fringes of long hairs on apical two-fifths on both antero- and postero-ventral aspects ; hind femora again have much long pubescence, and on antero-ventral aspect a row of strong variable bristles, missing on basal quarter, and on postero-ventral aspect a few long fine bristles towards apex, but not clearly distinguishable from the long fringe-like pubescence ; hind tibiae have the antero- and postero-ventral fringes very highly developed, many of the hairs on the postero-ventral side half as long as the tibiae themselves, and strong and almost bristle-like.

♀. The female of this species is one of the most easily recognized of the genus on account of the unusual form and prominence of tergum 6, which is easily observed without dissection (for details of the structure see fig. 6). Viewed laterally it is seen to project beyond tergum 5 and to be triangular in form, this being due to the dorsal portion being widened and so produced considerably to form the upper side of the triangle, and the segment narrowing ventrally so that the hind margin slopes forward to form the lower side of the triangle. Below it unites with sternum 6 so as to form with it a practically continuous ' collar.' Sterna 6 and 7 are large and exposed, as shown in fig. 6, *a*; sternum 6 is very wide, with a row of 8 or 10 strong setae along the hind margin ; sternum 7 is roughly triangular in form, with about six fine setae at the extreme

tip. Tergum 6 is also unusual in form as seen from above, from which view likewise it appears triangular, but more sharply pointed, and in addition divided or slit along the whole dorsal aspect (dissection shows that it actually consists of two separate plates ; fig. 6, *d*). The apex of the ' triangle ' as seen sideways is rounded, and on the outer margin of each plate of tergum 6 at this point is a row of six or seven short setae ; the rest of this unusually developed tergum is practically free from any setae or hairs, and appears shining and bare. In all other respects this insect bears a close general resemblance to the females of the allied species. Compared with *carnaria* L. it has a rather wider frons, the frontalia especially being wider, fully double the width of orbits ; chaetotaxy much the same, except that the scutellar discals are set so far back as to appear almost marginal. LEG CHAETOTAXY. Front femora with usual postero-ventral row of bristles strong and complete ; mid femora with antero-ventral series consisting of about four long widely spaced bristles in basal half and a well-developed comb in apical half, and on postero-ventral aspect a similar row of long bristles extending basally for two-thirds of length and with a less developed apical comb ; hind femora with a complete but irregular row of widely separated bristles on antero-ventral aspect and about five only long thin basal bristles postero-ventrally. The sternites are not characterized by specially long pubescence, as in the ♂, and are much like *carnaria* L. in this respect.

NOTES. *Scoparia* is not a common species in this country, although it is possibly merely overlooked owing to its general resemblance to *carnaria* L. We know it from about a dozen localities, such as the New Forest, Farley Downs, Studland, the Norfolk Broads, Herefordshire and Oban. Böttcher says that it is one of the commoner species of the genus, and Lundbeck records it from all Europe up to South Sweden, and also from Turkestan. Aldrich records it from North America, but this form may prove to be specifically distinct, or at any rate a good geographical subspecies, as among other differences the terminal segments are partly red.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. The ♂ terminalia of *scoparia* are illustrated in fig. 5. Sternum 5 (fig. 5, *b*) has rather long narrow lateral processes, and is armed with long hairs. The anal cercus when seen in side view is a long curved pointed plate ; the ventral view of the two cerci are shown in fig. 5, f. The phallosome when seen in profile (fig. 5, *a*) has a characteristic claw-like process at the distal end, which, however, when viewed endways is seen to be an almost complete collar-like ring. The two parts of the paramere are illustrated in fig. 5, *a*, *d*.

♀. The ♀ terminalia of *scoparia* are illustrated in fig. 6. The raised edges of tergum 6 (already noted) form a striking character which renders identification easy. This character, together with the wide sixth sternum (fig. 6, *a*, *e*) and the divided sixth tergum, should enable anyone to recognize the ♀ *scoparia* almost at a glance. The signum is wanting.

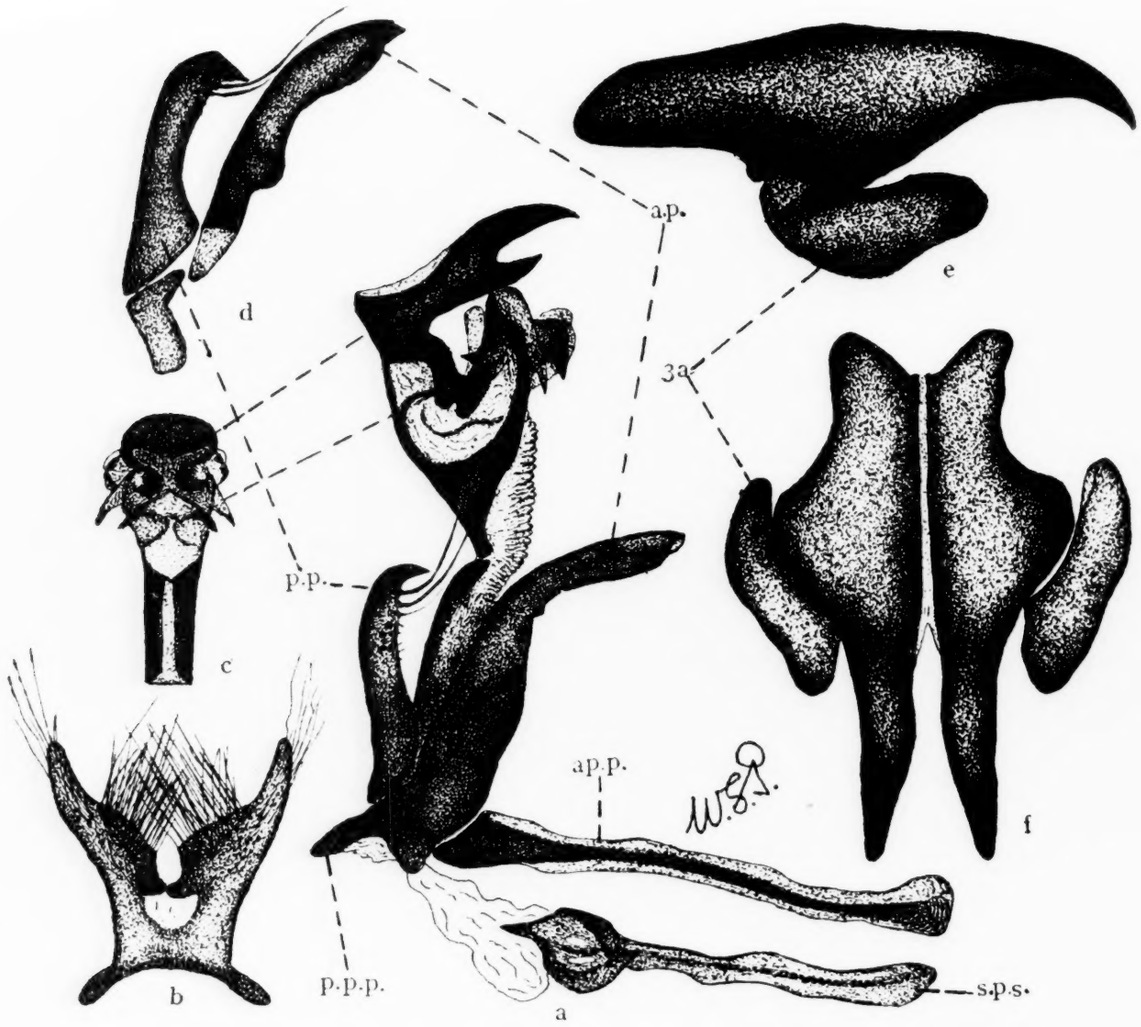


FIG. 5. *a*.—Phallosome and one paramere of *scoparia* in side view ; lettering as in fig. 1, *a* ; *b*.—Fifth sternum ; *c*.—Dorsal view of end of phallosome ; *d*.—Two parts of right paramere in side view ; *e*.—Lateral view of anal cercus and distal segment of ninth coxite ; *f*.—Ventral view of anal cerci and distal segments of ninth coxites.

Sarcophaga aratrix Pandellé. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂. The male of this species is one of the most easily and immediately recognizable, owing to the peculiar development of the apical (fifth) sternite, which is unlike that of any other British Sarcophaga. The basal portion is produced downwards and backwards as a conspicuous peak-like projection with a slightly bifid tip, and the whole sternite is bare and shining.

It is one of the larger species, bearing a general external resemblance to *carnaria* L., although not so large on the average, and in the keys it runs down near to the *carnaria* group. Like *carnaria* it has four or more D.C., the front ones being smaller than the two hinder ones ; undeveloped presutural acrs. ; bare first longitudinal wing vein ; black terminalia ; scutellum with a pair of fine apicals, strong discals and usually two other pairs of marginal bristles ;

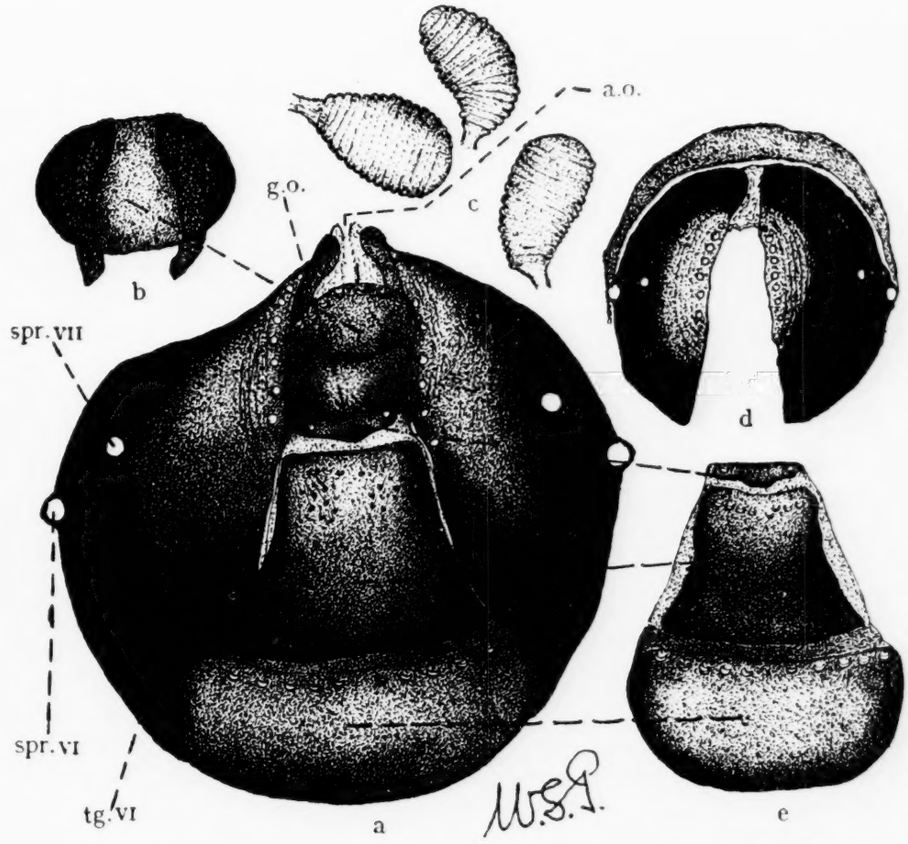


FIG. 6. *a*.—Ventral view of terminalia of ♀ *scoparia* to show diagnostic characters ; lettering as in fig. 2, *a* ; *b*.—Tenth sternum and anal cerci ; *c*.—Spermathecae ; *d*.—Sixth tergum showing its division into two parts ; *e*.—Sixth, seventh, ninth (?) sterna.

hind tibiae with long dense fringes of hairs antero- and postero-ventrally ; sternites with quite short, even pubescence, etc. It differs from *carnaria* in having usually no marginal setae on tergum 3 (apparent second), no marginal setae at all on tergum 7, and the chaetotaxy of the legs somewhat reduced.

Frons at narrowest about two-thirds eye-width ; third antennal joint about two-thirds as long again as second ; genal setae few, short and fine, not strongly developed ; small presutural intra-alars usually present ; well-developed prealars ; a pair of strong prescutellar acrs. ; no brush on lamellae of sternite 5, but a row of strong, thorn-like setae along margins ; tergum 6 with some silvery tomentum ; third costal segment nearly equal to fifth and sixth together. LEG CHAETOTAXY. Front femora have some long pubescence, especially ventrally at base, and a well-developed postero-ventral row of long bristles ; mid femora have also some long pilosity beneath basally, a complete antero-ventral row of short thorn-like setae, and a restricted similar postero-ventral row in apical half only ; no definite comb or ctenidium ; hind femora also with some long ventral pilosity, an antero-ventral row of strong long setae on apical two-thirds, no bristles on postero-ventral aspect, but a definite fringe of long hairs ; hind tibiae have antero- and postero-ventral fringes of long hairs.

♀. The female runs down in the keys to the *carnaria* L. group with four or five D.C., where, however, owing to the failure of the ♂ characters it falls to be compared with several species :—*carnaria* L. ; *vicina* Vill. ; *teretirostris* Pand. ; *scoparia* Pand. ; *albiceps* Mg. ; *tuberosa* Pand. ; and *similis* (Meade) Pand. Unfortunately we do not yet possess recognized ♀♀ of some of these species, and therefore cannot at present make anything like a complete comparative description. Fortunately we possessed two pairs of *aratrix* taken *in cop.*, so that we are nearly sure of their identity ; and we must be satisfied to describe them and compare them with such ♀♀ of allied species as we have. *Scoparia* Pand. will be easily separated through the characteristic terminal segments described above ; *tuberosa* Pand. var *exuberans* Pand., the only form so far taken in this country, has red terminalia. *Carnaria* L. and *vicina* Vill. usually have small marginals on second abdominal segment, and *aratrix* has none, but this is scarcely a reliable character ; a more reliable one seems to lie in the frontal width, etc. ; in *aratrix* the frons and genae are narrower than in the other two species ; in *aratrix* the genae at the narrowest part are barely half as wide as the eyes ; in *carnaria* and *vicina* about two-thirds ; in *aratrix* the frons at narrowest is barely equal to width of one eye ; in the other two species it is decidedly wider ; moreover, the facial tomentum in *aratrix* in the specimens examined appears to be silvery in colour, and in the other species golden, although it seems doubtful if these colours would be constant. The scutellar bristles seem more developed in *carnaria* and *vicina*, there being usually three well-developed ones on each side, and in *aratrix* two. This is again probably a variable character. Examination of some Indian and one Italian *albiceps* Mg. reveals differences. *Albiceps* is one of those species in which the chaetotaxy throughout tends to be reduced, more so in the Indian ones than in the Italian ; the presutural thoracic setae are small and the prescutellar acrs. fail, or are very small. There is a distinction, too, in the externally visible terminal segments ; in *albiceps* tergum 6 has a row of strong thick bristles along nearly the whole middle part of its margin, but failing for a space dorsally ; whilst in *aratrix* there are a number of rather crowded thinner bristles chiefly at the upper part of the tergum, and extending barely half way down, with short hairs only along the lower half of the margin.

Some Italian specimens which we believe to be ♀♀ of *tuberosa* Pand. resemble *albiceps* Mg. in the characters referred to above, and therefore differ from *aratrix* in the same manner. In them the frons is very wide—wider than in *albiceps* or *aratrix*. *Teretirostris* Pand. seems to resemble *aratrix* very closely in this sex ; comparison of a fairly reliable specimen of this species seems to show that *aratrix* might be recognized by its longer third costal section, which is longer than fourth and fifth together, whilst in *teretirostris* it is barely as long ; *aratrix* has no costal thorn, *teretirostris* a short one ; this one specimen of *teretirostris* at any rate has distinct hind marginals on second visible abdominal segment ; also numerous genal setae, whilst *aratrix* has only four or five ;

and we think it has stronger and more numerous bristles on tergum 6, but this is not clear. In these two species the terminalia are not produced externally enough to be easily understood without dissection.

Aratrix ♀ has frons at narrowest as wide as one eye ; orbits nearly as wide as frontalia ; antennae and chaetotaxy as in ♂, except that scutellar apicals fail. Tergum 5 has a row of strong marginal bristles; for tergum 6, see above. It is a species with silvery grey tomentum, and is thus blackish-looking, not brown ; sternites with very short pubescence ; no costal thorn. LEG CHAETOTAXY. Front femora with a full strong series of postero-ventral bristles, no antero-ventral series; mid femora on antero-ventral aspect usually with three short thick setae on basal half, and a fine comb of hair-like setae on apical

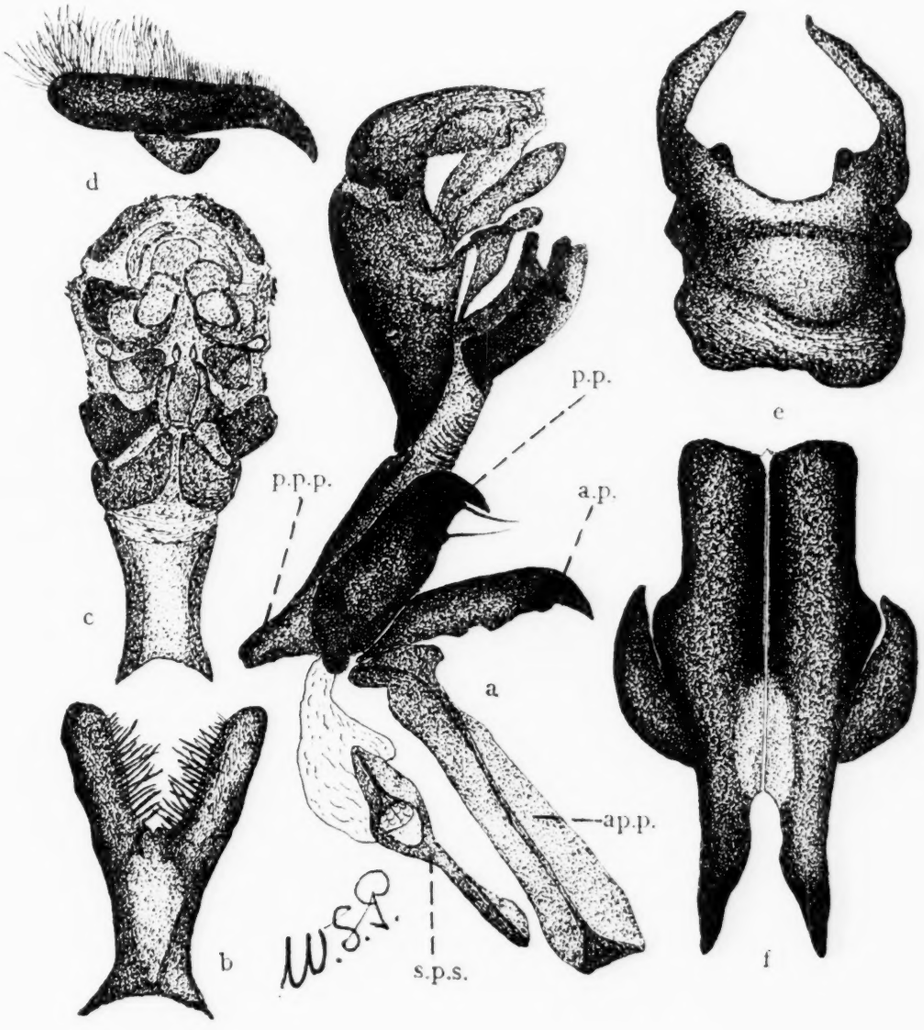


FIG. 7. *a*.—Phallosome and one paramere of *aratrix* in side view ; lettering as in fig. 1, *a* ; *b*.—Fifth sternum ; *c*.—Dorsal view of distal end of phallosome ; *d*.—Lateral view of anal cercus and distal segment of ninth coxite ; *e*.—Ninth tergo-sternum ; *f*.—Ventral view of anal cerci and distal segments of ninth coxites.

half, and on postero-ventral aspect there are about four longer strong setae on basal half and a similar rather less developed comb-like series towards apex ; hind femora have an almost complete but irregular series of moderate bristles on antero-ventral aspect ; and postero-ventrally only three or four solitary, long, strong bristles on basal portion.

NOTES. This is one of the less abundant British species, but is probably widely distributed, mixed with *carnaria* L. and *vicina* Vill., having similar habits of settling on the ground. On one occasion one of us found it in numbers near to Bewdley ; and we have records from various scattered localities chiefly in the Midlands and the South. It appears to occur in most European countries, Böttcher reporting it from Germany, Austria, France, Spain and Italy, and Lundbeck adding Denmark, Sweden and Finland.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. The ♂ terminalia of *aratrix* are illustrated in fig. 7. Sternum 5 (fig. 7, *b*) is markedly Y-shaped, with short, stout, bristle-like hairs on the inner sides of arms. The phallosome is long, and the distal end has five long processes and a hood-like one at the end ; these are shown in profile in fig. 7, *a* ; the dorsal view of the end of the phallosome is shown in fig. 7, *c*. The anal cercus in side view is seen as a simple curved

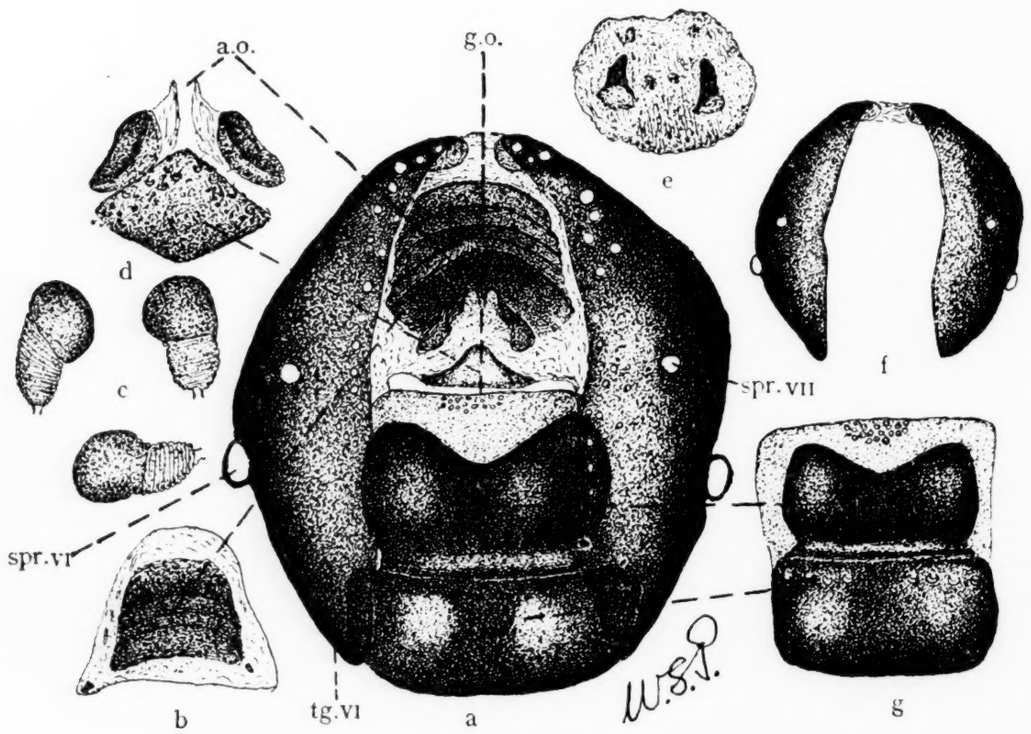


FIG. 8. *a*.—Ventral view of terminalia of ♀ *aratrix* to show diagnostic characters ; lettering as in fig. 1, *a* ; *b*.—Plates (? 7th, 9th terga) postero-dorsal to anal cerci ; these are very characteristic of this species ; *c*.—Spermathecae ; *d*.—Tenth sternum and anal cerci ; *e*.—Signum ; *f*.—Sixth tergum showing its division into two parts ; *g*.—Sixth, seventh, ninth (?) sterna.

plate, the end rather acutely bent and terminating in a point ; the ventral view of the anal cerci is shown in fig. 7, *f*.

♀. The ♀ terminalia of *aratrix* are illustrated in fig. 8. Tergum 6 in this species also consist of two plates, and the space postero-dorsal to the anal cerci is occupied by a ridged plate which is shown separately in fig. 8, *b* ; this plate, which is usually somewhat depressed, affords a good diagnostic character. There is a wide membraneous area distal to sternum 7, with a group of small hairs in the middle. The signum (fig. 8, *e*) is incompletely developed.

(*To be continued*)

THE OCCURRENCE OF TROPICAL TYPHUS IN SIERRA LEONE: A PRELIMINARY REPORT

BY

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AND

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(*Received for publication* 26 *May*, 1936)

During recent years fevers of the typhus group have been recorded from most parts of the world, and in Africa their presence has been noted in the north, south and east, the distribution of such cases as have been confirmed by the Weil-Felix reaction being shown on the accompanying map. It is surprising, therefore, that so few references are to be found regarding the diseases in West Africa, for in the literature available only five papers have been noted which concern typhus fevers in this last area; the results obtained by the authors are briefly quoted here. The Gold Coast report of the Laboratory Services for 1929-30 records negative results from agglutination reactions with *Proteus* X19 on 100 apparently unselected sera which had been submitted for the Wassermann reaction. Neujean (1934) in the Belgian Congo tested 104 sera (100 from healthy natives) against *Proteus* X19 and OX19, and in only one instance, that of an apparently healthy native, was a reaction in over 1 : 160 obtained; he therefore concludes that no pathological significance can be assigned to the few positive reactions obtained. Van Slype (1933) records a probable case of boutonneuse fever in a European who had been eight years in the Congo, but the Weil-Felix reaction was not carried out to confirm the diagnosis. More recently Cullen (1935) has reported a case of tropical typhus in Nigeria, in which the diagnosis was made on clinical grounds unsupported by laboratory confirmation. It is true that Daco (1935) has recorded an epidemic of typhus exanthematicus in the Belgian Congo, but, as is shown in the attached map, these cases all occurred in the Urundi Province, near a village called Kitega not far from the Tanganyika border.

In view, therefore, of the lack of confirmation of the two cases of tropical typhus recorded from Nigeria and the west of the Belgian Congo respectively, and of the purely negative evidence so far adduced by laboratories as to the occurrence of fevers of the typhus group in West Africa, it is of interest to record their presence in Sierra Leone.

Since May, 1935, the Weil-Felix reaction has been carried out on all sera submitted to the laboratory from cases of unexplained pyrexia in the hospital in Freetown, three strains of *Proteus* having been used, viz., OX19, OX2 and OXK. During the following 10 months 45 cases of fever of doubtful origin have had one or more Weil-Felix tests carried out, of which 5 cases have yielded

DATE OF ADMISSION 23.VII.35. NAME J.D. AGE 38 CASE No. I.

DATES OF OBSERVATION	23	24	25	26	27	28	29	30
DAYS OF DISEASE	6	7	8	9	10	11	12	13
TEMPERATURE FAHRENHEIT	A.M. 2 6 10 P.M. 2 6 10	A.M. 2 6 10 P.M. 2 6 10	A.M. 2 6 10 P.M. 2 6 10	A.M. 2 6 10 P.M. 2 6 10	A.M. 2 6 10 P.M. 2 6 10	A.M. 2 6 10 P.M. 2 6 10	A.M. 2 6 10 P.M. 2 6 10	A.M. 2 6 10 P.M. 2 6 10
104°								
103°								
102°								
101°								
100°								
99°								
98°								
97°								
Pulse per Minute	/ / 104/104	100/88 92/96 88	84/80 88/88 92	88/84 80/92 88	88/84 88/96 88	84/84 84/88 84	88/96 88/92 80	76/76 80/84 76

results positive in titres higher than 1 : 100 ; these positive results were obtained with *Proteus* OX19 and OX2, OXK never showing a reaction in higher dilution than 1 : 80.

Various antigens have been used by us in the course of our work, but all the results referred to in the present paper were attained when using alcoholic suspensions of OX19, OX2 and OXK, supplied by the Standards Laboratory, Oxford, the final reading recorded being that given after 12 hours' incubation at 37° C., followed by 12 hours at bench temperature. Dr. A. D. Gardner, who has kindly examined our findings, points out that the somewhat low titres recorded in our cases are due to incubating the suspensions at 37° C., instead of at 50° C., and that if the higher temperature technique had been adopted the titres of the positive sera would probably have read about twice as high.

Three of the five cases giving increased agglutination titres appeared on clinical and serological grounds to be suffering from tropical typhus ; the remaining two cases left hospital when symptoms abated and were not available for further serological investigation, so that, although their clinical condition was suggestive of tropical typhus, the diagnosis could not be established. A short account of the three cases diagnosed is given here, but all were African natives, and, as it was impossible to obtain an accurate history of the illness, or any detailed account of the symptoms from these patients, the clinical notes are of necessity very brief.

Case 1. J.D. Sudanese ; male ; aged 38 years ; occupation, cook. He has lived in Freetown for three years.

He stated on arrival at hospital on Aug. 23rd, 1935, that he had been ill for 5 days ; his temperature was found to be 104° F., and he was admitted. A copy of his 4-hourly temperature-chart for the first 8 days in hospital is figured here.

Laboratory findings :—

1935, Aug. 23rd (6th day of illness). Urine : a trace of albumen. Thick blood film : no malaria parasites. Faeces : Taenia ova and segments of *T. saginata*.

Aug. 24th (7th day of illness). Total white-cell count : 4,600 per c.mm. Differential count : polymorphonuclears—45·5 per cent. ; lymphocytes—53·0 per cent. ; monocytes—0·5 per cent. ; eosinophiles—1·0 per cent.

Blood cultured on this date in nutrient broth and in sodium taurocholate broth remained sterile during 7 days' incubation.

Widal and Weil-Felix reactions negative in all dilutions from 1 : 40 to 1 : 640.

Sept. 6th (20th day of illness). Widal negative in all dilutions as before.

Weil-Felix (only the maximum titres obtained will be quoted in these records) : OX19 = 1 : 160, + ; OX2 = 1 : 320, + ; OXK = 1 : 40, a trace.

Sept. 13th (27th day of illness). Weil-Felix : OX19 = 1 : 160, + ; OX2 = 1 : 640, + ; OXK = 1 : 160, negative.

Case 2. J.J. Sierra Leonean ; female ; aged 40 years ; petty trader. She has lived in Freetown all her life.

Felt ill first on Feb. 20th, 1936, with headache and fever ; this continued, and two days later she attended hospital and was admitted with a temperature of 104° F. and pulse 130. She was treated with intramuscular quinine (4 injections of 9 gr.) without her temperature responding. Her general condition improved steadily after admission, though her temperature did not begin to fall for some 9 days.

This patient's main symptoms were malaise associated with fever, burning pain in the eyes and violent frontal headache ; nausea and vomiting were present to a slight degree

for a few days. Examination revealed little, apart from congestion of the conjunctivae and a few raised papules on the abdomen and extensor surfaces of the forearms ; her spleen was enlarged two fingers.

Laboratory findings :—

1936, Feb. 23rd (4th day of illness). Thick blood film ; no malaria parasites found.

Feb. 29th (10th day of illness). Widal negative in all dilutions from 1 : 40 to 1 : 640. Weil-Felix : OX19 = 1 : 320, + ; OX2 = 1 : 40, + ; OXK = 1 : 40, +.

March 3rd (13th day of illness). Weil-Felix : OX19 = 1 : 1,280, + ; OX2 and OXK negative in titres 1 : 80 to 1 : 1,280.

March 10th (20th day of illness). Weil-Felix : OX19 = 1 : 3,200, + ; OX2 = 1 : 80, + ; OXK failed to react in titres 1 : 40 to 1 : 640.

Case 3. D.K. Sierra Leonean ; female ; aged 26 years ; teacher. She has lived in Freetown all her life.

Illness commenced suddenly on evening of March 15th, 1936, with chill (though no rigor occurred), severe headache over the vertex, and eyes aching and congested. This condition persisted for a week, when she went to a doctor. She stated that her headache was better but that she felt very weak ; her temperature was 101° F., but examination revealed nothing to explain the pyrexia. A thick blood film was sent to the laboratory to be examined for malaria parasites, and the patient was given quinine ; no parasites were found in the film. On March 25th, she again visited the doctor, when she stated that she felt ' very collapsed ' ; since her temperature was 104° F. and she appeared very toxic, she was admitted to hospital immediately. During the next two days her temperature varied between 99° F. and 102° F., and then subsided, together with her symptoms. She never observed any rash and none was noted during her stay in hospital.

Laboratory findings :—

1936, March 16th (2nd day of illness). Examination of thick blood film revealed no malaria parasites.

March 25th (11th day of illness). Thick blood film examined for malaria parasites with negative result.

Faeces examined for ova and pathogenic protozoa with negative results.

March 26th (12th day of illness). Widal negative in dilutions from 1 : 40 to 1 : 640.

Weil-Felix : OX19 = 1 : 3,200, + ; OX2 = 1 : 40, + ; OXK negative in dilutions from 1 : 40 to 1 : 640.

March 31st (17th day of illness). Weil-Felix : OX19 = 1 : 6,400, + ; OX2 and OXK negative in dilutions from 1 : 40 to 1 : 640.

The diagnosis of typhus fever in these cases was based on the following points : the abrupt onset, the high fever with daily remissions and decline by lysis in 10–14 days, the severe and persistent headache, the development of a positive Weil-Felix reaction as the pyrexia and symptoms abated, and the high agglutination titres eventually obtained with the *Proteus* strains. It is of interest to note, in addition, the following points : all three cases showed during the greater part of their illness a relatively slow pulse associated with the pyrexia ; none showed or gave a history of a primary lesion associated with the illness ; the characteristic rash was not observed, probably owing to the difficulty of seeing it against the black skin.

The three cases we have just described represent the first conclusive proof of the existence of typhus fever in West Africa. We are of the opinion, however, that the disease is probably more widespread and more common in West Africa than the previous lack of evidence would lead us to believe, and that if routine Weil-Felix reactions were carried out on selected cases of



MAP OF AFRICA

Showing, by shading, the areas from which proved cases of typhus have been recorded. Until the occurence of cases in Sierra Leone, described in the present paper, the disease appeared to be confined to north, east and south Africa.

unexplained pyrexia a proportion of them would be found to be due to typhus. Now that standardized killed suspensions of the various types of *Proteus* X are readily available, the carrying out of the reaction presents little more difficulty than that of an ordinary Widal.

An examination of the available literature regarding epidemics of pyrexial diseases, occurring in West Africa and bearing a resemblance to typhus, reveals that such outbreaks have occurred with considerable frequency during recent years. As early as 1917, Le Fanu (1921) reported from the Gold Coast a series of cases amongst Europeans and Africans which, he believed, bore a resemblance to dengue fever ; these cases were associated with raised temperature, a relatively slow pulse, headache, joint pains and an erythematous rash. From that time onwards similar cases have been reported at various times from the same district. Davies and Johnson (1921), working in Nigeria, have described a disease with similar symptoms which they believed also belonged to the ' dengue group.' It is interesting to note that Ross (1932), in his account of an outbreak of typhus at Salisbury in Southern Rhodesia, comments on the similarity between his cases and those described by Davies and Johnson in Nigeria. In addition to the work of the English doctors in Nigeria and the Gold Coast, the French medical press contains numerous references to epidemics of fever of a dengue type which have occurred in the French and Belgian West African colonies, especially in the Belgian Congo, where they appear to be concentrated in the neighbourhood of Leopoldville. Thus Suldey (1926), Legendre (1926, 1928), Lefrou (1927, 1928), Aubin (1930) and Pieraerts (1931*a*, 1931*b*) have all published accounts of these epidemics of unknown origin. These writers are not all agreed as to how closely these epidemics resemble classical dengue : some of them give them the title of ' pseudo-dengue ou fièvre rouge congolaise ' ; others regard the ' fièvre rouge congolaise ' as a distinct disease. All the records examined by us, however, show a close resemblance in their descriptions of the signs and symptoms of the disease, not only to each other, but also to the cases described by the English writers from the Gold Coast and Nigeria ; and these, in their turn, have been shown by Ross (1932) to resemble the proved cases of tropical typhus occurring in Rhodesia.

The Weil-Felix reaction does not appear to have been investigated in any of these West African epidemics, so that we are unable to state the likelihood of their having been caused by a virus of the typhus group. On the other hand, their resemblance to cases of typhus reported from other parts of Africa, and our own discovery of a focus of the disease in Sierra Leone, suggests the necessity for a fuller investigation of the part played by tropical typhus in causing disease in West Africa.

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THE INFLUENCE OF REPEATED TRANSMISSIONS IN ANIMALS ON THE VIRULENCE OF *TRYPANOSOMA RHODESIENSE* AND *TRYPANOSOMA BRUCEI*

BY

J. F. CORSON

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It is generally agreed that polymorphic trypanosomes, when maintained for several years by direct passages through small laboratory animals—especially if only one species of animal is used—lose some of their distinctive characters. The chief characters of polymorphic trypanosomes are polymorphism, ability to produce posterior-nuclear forms, virulence, reaction, *in vitro* and *in vivo*, to normal human blood-serum and blood-plasma, reaction to certain chemicals, characteristic development in tsetse-flies, and, in the case of the human trypanosomes, infectivity to man. As would be expected, there is much variation, among different strains and in different circumstances, in the changes that may take place. Most frequently it is found that polymorphism and the production of posterior-nuclear forms disappear, at first temporarily and later, in at least some strains, probably permanently. The susceptibility of strains of *T. rhodesiense* to human serum increases with increase of time since isolation from man, though individual trypanosomes may retain their resistance for at least many months. The power to develop in tsetse-flies, which seems to resemble the power to develop in artificial culture media, as was pointed out by Thomson and Sinton (1912) and later by Reichenow (1934), was retained, in the case of the 'Hamburg alt' strain of *T. brucei*, after direct passages in Europe, for ten years (Schilling and Schreck, 1930); and in the case of another strain of *T. brucei* (Corson, 1932; Duke, 1934) for over four years. On the other hand, Duke (1934) has failed to infect flies with more recently isolated strains. To what extent these changes in the character of trypanosomes are dependent on direct transmission and association with one species of vertebrate host, is unknown. Duke (1928) made experiments on the effects of repeated direct transmissions on various strains of polymorphic trypanosomes, and found that 'in each case, after a year or so, the strain—easily transmissible on its first isolation—lost its power to develop in tsetse, and changes in morphology and virulence also took place.' He goes on to say, however: 'I now know that the changes which appeared after two years or more of direct transmission, involving some thirty passages, may occur, for reasons at present unknown, at any moment in the life of a strain, with or without the operation of direct transmission. In other words, the transmissibility of a trypanosome is a very variable and unstable character.' Referring to direct transmission he adds: 'It is evident

from experiment that this method may function for a long time without producing any effects at all on the strain.' The question of the loss of infectivity to man by the human trypanosomes is naturally a difficult one. By analogy with *T. brucei* it is reasonable to think that some strains of human trypanosomes can infect some men, but not others who are more resistant, and therefore that a complete loss of infectivity to man is possible. Duke (1935*a*) concluded that one strain of *T. rhodesiense*, strain Tinde I, had lost its infectivity to man after a number of direct passages, interspersed with fly passages, through rats, monkeys and antelopes ; and that another strain of *T. rhodesiense*, strain Tinde II, showed infectivity to some men but not to others, after similar treatment ; and that a third strain of *T. rhodesiense*, strain Tinde III, had lost its infectivity to man as a result of a series of direct passages through 14 guinea-pigs during about 18 months. On the other hand, a strain of *T. rhodesiense*, strain Bupamba (Corson, 1932), retained both its transmissibility by tsetse-flies and its infectivity to man after 20 direct passages, comprising 14 through goats, then 2 through rats, then 1 through a goat and 3 through sheep. Fairbairn (1933) found that his strain of *T. rhodesiense*, no. 26, which was the same as strain Tinde III of Duke, retained its infectivity to man after 19 direct passages through rats, during about 11 months after isolation from man. With regard to virulence, a series of many direct passages through rats or mice in laboratories has usually resulted in an increase in virulence. Thomson and Robertson (1926) found that *T. rhodesiense*, strain M, ' very soon attained a fixed virulence which has been steadily maintained ever since.' The period occupied by the 40 transmissions is not given, but it seems to have been about two or three years. One strain of *T. brucei* (Corson, 1934) developed greatly increased virulence after latency for several months in a rat of the second experimental passage, and the increase in virulence was maintained in further direct passages. It was passed through *Glossina morsitans* and the virulence for rats was not diminished, but it failed to infect man. It is clear that there is very little knowledge of how these changes are caused and that much more study is needed. As in the case of malaria or yellow fever, much of the work could probably be done best in the laboratories of Europe if it could be carried out in circumstances that are free from the objection that the experiments, or control experiments, do not sufficiently represent what is thought to occur in nature in tropical Africa. It is not now very difficult to send pupae of tsetse-flies and recent strains of trypanosomes to Europe ; and it may be found that continuous cyclical transmission is not necessary for the preservation of the characters of the trypanosomes.

With this idea in mind, it was thought that it might be useful to examine records of transmissions of some freshly isolated strains of *T. rhodesiense* and *T. brucei*. As polymorphism and the production of posterior-nuclear forms were not lost in any of the strains personally studied, which include all the strains except two, attention was limited to virulence, and the duration of life after infection was taken as a rough measure of virulence for a species of animal.

In the case of one strain, *T. rhodesiense*, Kibondo, transmissions were made by single isolated *G. morsitans*, except the first passage, and rats were infected from time to time by these single flies, so that the date of their infection was known. Three of the strains were transmitted through goats and sheep, while occasional passages through guinea-pigs, birds and Hyrax appear in the records. When transmissions were made through rats only, there was an increase in virulence in most strains, but this was not evident in sheep and goats. In the strain, *T. rhodesiense*, Kibondo, there was no clear indication of any stabilizing action of repeated fly-passages, as judged by the infection in test rats ; and this agrees with the rather surprising results of several experiments which show that one or two passages through tsetse-flies, after a number of direct transmissions, do not seem to alter the trypanosomes in regard to their virulence, morphology, reaction to human serum and resistance to drugs. The various strains are shown in the Table. The strain, *T. brucei* vel *rhodesiense* of Bruce and his co-workers (1915), strain XXVI, was isolated by them from a naturally infected dog in Nyasaland in 1913, and the transmissions are copied from their Table XCIX. The list of transmission of *T. rhodesiense*, strain M, of Thomson and Robertson (1926), is compiled from their chart, and the duration of life of the rats is estimated as nearly as possible from the chart.

SUMMARY

The influence on polymorphic trypanosomes of maintenance by direct passages, as used in laboratories where tsetse-flies could not be obtained, is discussed, and it is suggested that little is known about the subject.

Records of transmissions of several recent strains of *T. rhodesiense* and *T. brucei* are given in a Table, the duration of life after infection being regarded as a rough measure of the virulence of the trypanosomes.

TABLE

Strain	Date of inoculation	Inoculated animal	Infected from	Duration of life : days
T. rhodesiense	25. 3.30	Goat 14	Man	36
Bupamba, of	30. 4.30	„ 18	Goat 14	40
Maswa, T.T.	24. 5.30	„ 21	„ 18	18
	10. 6.30	„ 32	„ 21	31
	24. 6.30	„ 40	„ 32	22
	6. 7.30	„ 47	„ 40	31
	30. 7.30	„ 53	„ 47	20
	17. 8.30	„ 68	„ 53	32
	18. 9.30	„ 86	„ 68	33
	16.10.30	„ 16	„ 86	39
	14.11.30	„ 102	„ 16	46
	13.12.30	„ 121	„ 102	31
	10. 1.31	„ 127	„ 121	35
	6. 2.31	„ 136	„ 127	?
	21. 2.31	Rat 197	„ 136	23
	2. 3.31	„ 204	Rat 197	34
	23. 3.31	Goat T2	„ 204	over 49
T. rhodesiense	15. 2.30	Rat 1	Man	—
Masunga	24. 3.30	Goat 4	Rat 1	43
Longsiku of	2. 5.30	„ 25	Goat 4	38
Maswa	9. 6.30	„ 29	„ 25	36
	6. 7.30	„ 49	„ 29	29
	28. 7.30	„ 62	„ 49	32
	17. 8.30	„ 72	„ 62	34
	16. 9.30	„ 80	„ 72	34
	17.10.30	„ 94	„ 80	39
	4.11.30	„ 105	„ 94	53
	24. 3.30	Sheep 6	Rat 1	50
	6. 5.30	„ 21	Sheep 6	55
	13. 6.30	„ 31	„ 21	30
	24. 6.30	„ 44	„ 31	45
	28. 7.30	„ 66	„ 44	50
	16. 8.30	„ 68	„ 66	44
	17. 9.30	„ 92	„ 68	69
	18.10.30	„ 46	„ 92	—
	12.12.30	„ 118	„ 46	65
	22. 9.30	Rat 72	„ 68	—
	27.10.30	„ 85	Rat 72	—
	20.11.30	Fowl 5	„ 85	—
	6. 1.31	„ 2	Fowl 5	—
	18. 1.31	Rat 163	„ 2	30
	1. 2.31	Francolin 5	Rat 163	—
	19. 2.31	Rat 179	Francolin 5	33

TABLE—(*Continued*)

Strain	Date of inoculation	Inoculated animal	Infected from	Duration of life : days
T. rhodesiense Lutambi of Maswa, T.T.	16. 3.30	Rat 3	Man	—
	6. 4.30	Goat 8	Rat 3	39
	12. 5.30	„ 27	Goat 8	33
	13. 6.30	„ 31	„ 27	33
	24. 6.30	„ 38	„ 31	30
	6. 7.30	„ 43	„ 38	44
	17. 8.30	„ 74	„ 43	38
	16. 9.30	„ 83	„ 74	56
	17.10.30	„ 97	„ 83	over 46
	14.11.30	„ 108	„ 97	44
	13.12.30	„ 120	„ 108	34
	10. 1.31	„ 126	„ 120	over 51
T. rhodesiense Masunga Lambo of Maswa	11. 6.31	Rat 254	Man	27
	24. 6.31	Hyrax 17	Rat 254	—
	8. 8.31	Rat 329	Hyrax 17	23
	21. 8.31	„ 343	Rat 329	20
	7. 9.31	Francolin T4	„ 343	—
	15. 9.31	Rat 364	Francolin T4	16
	23. 9.31	Francolin T14	Rat 364	—
	1.10.31	Rat 396	Francolin T14	15
	16.10.31	„ 427	Rat 396	11
	23.10.31	„ 443	„ 427	10
	7. 9.31	Francolin T6	Rat 343	—
	15. 9.31	Rat 370	Francolin T6	14
	23. 9.31	Francolin T15	Rat 370	—
	1.10.31	Rat 397	Francolin T15	9
	23. 9.31	Francolin T16	Rat 376	—
	1.10.31	Rat 398	Francolin T16	15
T. rhodesiense Wosuka of Maswa	17.10.30	Rat 81	Man	48
	20.11.30	Fowl 1	Rat 81	—
	31.12.30	Rat 144	Fowl 1	35
	12. 1.31	Guinea-fowl	Rat 144	—
	27. 1.31	Rat 176	Guinea-fowl	32
	26. 2.31	„ 201	Rat 176	23

TABLE—(*Continued*)

Strain	Date of inoculation	Inoculated animal	Infected from	Durations of life : days
T. rhodesiense Nangiwa of Ushirombo, T.T.	12. 5.31	Rat U2	Man	47
	4. 6.31	„ U2a	Rat U2	43
	3. 7.31	„ 267	„ U2a	39
	12. 7.31	„ 275	„ 267	40
	1. 8.31	„ 326	„ 275	30
	26. 8.31	„ 346	„ 326	19
	6. 9.31	„ 358	„ 346	22
	27. 9.31	„ 388	„ 358	13
	9.10.31	„ 424	„ 388	15
	19.10.31	„ 436	„ 424	15
	25.10.31	„ 447	„ 436	31
	7.11.31	„ 453	„ 447	14
	15.11.31	„ 459	„ 447	29
	29.11.31	„ 477	„ 459	34
	23.12.31	„ 501	„ 477	34
	2. 1.32	„ 515	„ 501	?
	21. 1.32	„ 531	„ 515	21
	3. 2.32	„ 538	„ 531	17
T. rhodesiense Kanawata of Ushirombo	12. 5.31	Rat U1	Man	48
	4. 6.31	„ U1a	Rat U1	40
	3. 7.31	„ 266	„ U1a	38
	28. 7.31	„ 316	„ 266	49
	30. 8.31	„ 349	„ 316	49
	19. 9.31	„ 378	„ 349	13
	28. 9.31	„ 394	„ 378	49
	19.10.31	„ 435	„ 394	32
	25.10.31	„ 446	435	18
	15.11.31	„ 458	„ 394	?
	24.11.31	„ 470	„ 458	?
	17.12.31	„ 491	„ 470	37
	29.12.31	„ 509	„ 491	36
	25. 1.32	„ 535	„ 509	11
	28. 1.32	„ 536	„ 509	15
T. rhodesiense Solezi of Ushirombo	3. 5.31	Rat RU21	Man	—
	24. 6.31	„ RU54	Rat RU21	—
	13. 7.31	„ 281	„ RU53	—
	27. 7.31	„ 305	„ 281	43
	26. 8.31	„ 347	„ 305	46
	4.10.31	„ 404	„ 347	20
	19.10.31	„ 438	„ 404	26
	25.10.31	„ 444	„ 438	22
	15.11.31	„ 461	„ 444	7
	22.11.31	„ 468	„ 461	10
	29.11.31	„ 473	„ 468	12
	10.12.31	„ 489	„ 473	7
	14.12.31	„ 490	„ 489	9
	23.12.31	„ 500	„ 490	9

TABLE—(*Continued*)

Strain	Date of inoculation	Inoculated animal	Infected from	Duration of life : days
T. rhodesiense	2. 7.31	Rat RU58	Man	—
Paulo of	13. 7.31	„ 282	Rat RU58	—
Ushirombo	27. 7.31	„ 307	„ 282	65
	6. 9.31	„ 357	„ 307	34
	4.10.31	„ 405	„ 357	34
	25.10.31	„ 445	„ 405	26
	7.11.31	„ 454	„ 445	42
	17.12.31	„ 492	„ 454	15
	29.12.31	„ 510	„ 492	23
	7. 1.32	„ 520	„ 510	18
	21. 1.32	„ 532	„ 520	45
	3. 2.32	„ 537	„ 532	19
	22. 2.32	„ 550	„ 537	13
	5. 3.32	„ 551	„ 532	19
	12. 3.32	„ 552	„ 551	15
T. rhodesiense, M.		Rat 1	Man	75
(Thomson &		„ 2	Rat 1	50
Robertson, 1926)		„ 3	„ 2	62
		„ 4	„ 3	41
		„ 5	„ 4	20
		„ 6	„ 5	11
		„ 7 & 8	„ 6 & 7	29
		„ 9 & 10	„ 8 & 9	18
		„ 11	„ 10	28
		„ 12	„ 11	25
		„ 13–15	„ 12–14	13
		„ 16	„ 15	20
		„ 17	„ 16	16
		„ 18–26	„ 17–25	15–20
		„ 27	„ 26	9
		„ 28	„ 27	17
		„ 29–40	„ 28–39	8–11
T. rhodesiense	24. 7.33	Guinea-pig 95	Man	—
Kibondo, T.T.		*G. morsitans* b	Guinea-pig 95	
		„ x	„ 95	
	23. 9.33	Rat 908	Fly x	28
	28. 9.33	„ 926	„ x	50
	28. 9.33	„ 927	„ x	40
	18.10.33	„ 951	„ b	32
		Dikdik	„ x	
		Fly K8	Dikdik	
		Dikdik	Fly K8	
		Fly S8	Dikdik	

TABLE—(*Continued*)

Strain	Date of inoculation	Inoculated animal	Infected from	Duration of life : days
T. rhodesiense	18. 2.34	Rat 1091	Fly S8	29
Kibondo, T.T.	9. 3.34	„ 1163	„ S8	16
(*con.*)	13. 3.34	„ 1164	„ S8	23
	29. 3.34	„ 1172	„ S8	15
		Dikdik	„ S8	
		Fly AA53	Dikdik	
	4. 5.34	Rat 1179	Fly AA53	27
	15. 5.34	„ 1303	„ AA53	27
	18. 5.34	„ 1312	„ AA53	26
		Dikdik	„ AA53	
		Fly AL27	Dikdik	
		„ AL41	„	
	12. 9.34	Rat 1499	Fly AL27	26
	7. 9.34	„ 1389	„ AL41	20
	17. 9.34	„ 1452	„ AL41	32
		Reedbuck	Flies AL27 & AL41	
		Flies series		
		AS, AT, AU	Reedbuck	
	31.10.34	Rat 1480	Fly AU20	26
	31.10.34	„ 1549	„ AU 1	20
	31.10.34	„ 1584	„ AU19	13
	31.10.34	„ 1554	„ AT34	22
	31.10.34	„ 1572	„ AT27	18
	31.10.34	„ 1573	„ AT 1	27
	31.10.34	„ 1490	„ AS 7	19
	31.10.34	„ 1588	„ AS 6	13
	31.10.34	„ 1471	„ AS 12	12
T. brucei vir.	29. 6.31	Guinea-pig T11	Guinea-pig T9	34
From Mr. Hornby.	12. 7.31	„ T13	„ T11	25
From cow early in	29. 7.31	„ T23	„ T13	29
1927, then 50 direct	9. 8.31	„ T25	„ T23	26
passages in rats,	4. 9.31	„ T31	„ T25	43
guinea-pigs, rab-	19.10.31	„ T35	„ T31	30
bits and goats, to	11.11.31	„ T38	„ T35	23
guinea-pig T9	25.11.31	„ T40	„ T38	31
	26. 8.31	„ T29	„ T23	41
	7.10.31	„ T34	„ T29	35
	11.11.31	„ T37	„ T34	26

TABLE—(*Continued*)

Strain	Date of inoculation	Inoculated animal	Infected from	Duration of life : days
T. brucei mild		Rat 262	Rat	
From Mr. Hornby.	24. 6.31	„ 263	Rat 262	26
From an ox in Oct.,	29. 6.31	„ 265	„ 263	32
1930	27. 7.31	„ 303	„ 265	18
	1. 9.31	„ 353	„ 303	39
	16. 9.31	„ 374	„ 353	36
	27. 9.31	„ 390	„ 374	43
	27.10.31	„ 449	„ 390	43
	20.11.31	„ 464	„ 449	42
	20.12.31	„ 495	„ 464	37
T. brucei of Zululand	6. 9.32	Dog	Donkey	
From Dr. du Toit.	3.10.32	Rat 601	Dog	134
Isolated from a	4. 2.33	„ 687	Rat 601	72
naturally infected	15. 4.33	„ 775	„ 687	killed
donkey	5. 6.33	„ 787	„ 775	„
	15. 7.33	„ 834	„ 787	67
	12. 8.33	„ 861	„ 834	73
	11. 9.33	„ 884	„ 861	82
	26.10.33	„ 966	„ 884	75
	10.12.33	„ 1056	„ 966	54
	13.10.32	„ 607	Dog	254
	24. 6.33	„ 814	Rat 607	17
Virulent branch	8. 7.33	„ 825	„ 814	18
	19. 7.33	Guinea-pig 92	„ 825	72
	28. 8.33	Rat 865	Guinea-pig 92	19
	13. 9.33	„ 886	Rat 865	19
	30. 9.33	„ 943	„ 886	23
	22.10.33	„ 964	„ 943	17
	4.11.33	„ 971	„ 964	14
	14.11.33	„ 981	„ 971	13
	27.11.33	„ 1026	„ 981	12
	9.12.33	„ 1049	„ 1026	14
	21.12.33	„ 1068	„ 1049	11
	31.12.33	„ 1077	„ 1068	9
	8. 1.34	„ 1084	„ 1077	16
	15. 1.34	„ 1119	„ 1084	12
	26. 1.34	„ 1136	„ 1119	11
	3. 2.34	„ 1150	„ 1136	12
	15. 2.34	„ 1154	„ 1150	9

TABLE—(*Continued*)

Strain	Date of inoculation	Inoculated animal	Infected from	Duration of life : days
T. brucei vel *rhodesiense* Strain XXVI from naturally infected dog. (Bruce, 1915)	26. 3.13	Rat 311	Rat 67	18
	26. 3.13	,, 312	,, 67	60
	30. 3.13	,, 391	,, 67	21
	30. 3.13	,, 392	,, 67	21
	5. 4.13	,, 407	,, 312	54
	20. 4.13	,, 462	,, 407	25
	25. 5.13	,, 585	,, 407	28
	14. 6.13	,, 670	,, 585	27
	2. 7.13	,, 786	,, 670	45
	2. 8.13	,, 1020	,, 786	30
	30. 8.13	,, 1218	,, 1020	94
	19.10.13	,, 1492	,, 1218	54
	12.12.13	,, 1687	,, 1492	16
	28.12.13	,, 1719	,, 1687	32
	7. 1.14	,, 1741	,, 1719	15
	9. 1.14	,, 1749	,, 1719	18
	27. 1.14	,, 1814	,, 1749	16
	10. 2.14	,, 1855	,, 1814	20
	22. 2.14	,, 1945	,, 1855	17
	7. 3.14	,, 1985	,, 1945	20
	25. 3.14	,, 2023	,, 1985	18
	10. 4.14	,, 2070	,, 2023	12
	22. 4.14	,, 2105	,, 2070	20
	7. 5.14	,, 2124	,, 2105	8
	15. 5.14	,, 2168	,, 2124	17

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CERTAIN ASPECTS OF THE WELFARE OF WOMEN AND CHILDREN IN THE COLONIES

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INTRODUCTION

The early activities in the development of most of the colonies were chiefly the concern of men ; such activities, for example, as the maintenance of law and order, the development of trade, of transport and the utilization of natural resources. Men from the home countries generally originated these works, and it was the male members of the local populations who assisted in developing them.

As a result of this, the first schools which were built were for the education of boys, the hospitals were engaged chiefly in treating men patients, and in some cases, as in certain projects such as the construction of railways or mines, even the housing accommodation was sometimes for the use of male labourers alone.

In districts where missionaries were working conditions were frequently better, as the women missionaries took part in educational and medical work among the women and girls.

In colonies where a markedly one-sided state of affairs existed it was soon seen that serious results were arising, but when efforts were made to improve matters surprising difficulties were often encountered. Difficulty sometimes arose from the timidity of the local women and from their suspicion of strange foreign customs and institutions ; in countries where women lived in a more or less secluded position it was often found very difficult to get them to make use of educational or medical institutions when these were provided. Again, the

type of education given in earlier days in girls' schools was often considered by the people to be neither useful nor suitable, and the women were reluctant to attend hospitals staffed by men.

There were formerly in many of the colonies very few women from overseas who could take part in the work, and the health conditions of the country were often so bad that little encouragement was given to women to go out there.

The advent of women doctors, health visitors and nursing sisters, together with an improved curriculum in girls' schools, has caused improvement in these respects—an improvement, however, which has still far to go, and which, while fairly evident in some colonies, is almost non-existent in others.

There is to-day a considerable number of people who are dissatisfied with the progress made and who have been urging that more attention might be given to the welfare of women and children in the colonies.

Societies interested in the welfare of children have been distressed by the high rates of infant mortality, by the possible evils associated with systems of adoption in some colonies, and by a suspicion of the existence of forms of slavery, as in the system of ' muitsai ' now under investigation.

Societies interested in women's welfare have been concerned about the high rates for maternal mortality, by the great lack of education for girls, by the lack of attention to the training of local nurses, and by the insufficient investigation of certain social conditions which react unfavourably upon women.

Many missionary societies have been active in drawing attention to the position, while they have at the same time taken an energetic part themselves in medical work for women and children.

As a member of the Women's Medical Service for India, and later of the Colonial Medical Service in Africa, I have been able to learn something of the medical requirements of women and children in these countries. By means of a grant of a Leverhulme Fellowship I was enabled in the summer and autumn of 1935 to make a tour of the colonies of Hong Kong, Malaya, Ceylon and Palestine, and also to pay a short visit to China, Burma and India, to see something of the work which is being done there. In all these countries I have had many opportunities of discussing the problems with those engaged in this branch of medical work and have been able to study different methods of organization.

In the present report, I have tried to describe the present position throughout the colonies, to draw attention to some unsatisfactory aspects, and to explain how certain conditions seem to have arisen ; and I have also made suggestions as to how I think improvement might be effected. An account is given of several interesting recent developments in different countries, more particularly with regard to the training of health visitors, nurses and midwives, which I think are promising and worthy of study ; information on these matters is not always accessible to workers in the colonies.

While I have naturally drawn upon my own medical experience and upon what I have observed in my recent tour, it must be understood that to a great

extent this report is based on opinions expressed to me by doctors, health visitors and nursing sisters engaged in this work throughout the colonies.

For the purpose of this report I drew up Tables giving statistics of death-rates, infant and maternal mortality-rates, and numbers of out- and in-patients for the various colonies ; but I have now deleted these for several reasons.

Firstly, because a comparison of these figures gives an entirely erroneous impression of the progress of the work of the respective colonies ; in a poor or recently acquired colony the figures may appear bad, and yet there may be in such a colony an energetic policy with regard to work among women and children.

Secondly, because accurate vital statistics can only be obtained from a very few colonies, and in those colonies where health conditions are worst we frequently find that few vital statistics are collected.

I also considered giving an account of the work as I had seen it in the various colonies, or had read of it in the reports ; but here again I felt that comparisons might be made which would be unjustifiable, as they would fail to take into consideration the great economic, cultural and educational differences between the various colonies. For these reasons the subject is reviewed here in a general way.

I. THE IMPORTANCE OF WELFARE WORK AMONG WOMEN AND CHILDREN

It may be asked : ' Can the well-being of women and children in a country, even from a medical point of view, be considered separately from that of the men ? ' The answer is that to a great extent it cannot, and that, when it is considered too much as a separate subject, the women suffer. This arises partly because the administrators and purse-holders are generally men, and if they are not associated with the work among women they will not realize its importance, and improvements which require legislation and expenditure will be delayed. Again, the well-being of women and children demands the active co-operation of the father of the family, and if he is not given information, and if his interest and sympathy are not enlisted in the work, he may be neglectful of, or even antagonistic to, very necessary measures for improvement. For the purpose of improving health among a people it is often advisable to consider them more as an aggregate of family units than as so many men and so many women.

While bearing the above in mind, I consider that there still remain certain aspects of the well-being of women and children which can best be considered separately. These arise because of the fact that the vast majority of women in the colonies are mainly occupied in looking after a home and a husband and in giving birth to and bringing up children. That this occupation has often to be combined with work in the farm or field, with plantation or factory labour,

with street- or shop-trading is true, but it is the first-mentioned factors which make their problems distinct.

In order to keep a home and to care for a family in a healthy manner, women require a special type of education ; special facilities must be provided so that they and their children may have adequate medical treatment ; special arrangements must be made so that they receive education in health matters ; and a special study must be made of the social and industrial conditions which may have harmful effects on their well-being.

Were these factors attended to, were the women given the knowledge and opportunity to keep a healthy home and family, the good effects would naturally be felt also by the men and so by the whole community.

One might think that it is unnecessary to state this, but recently there has been in a few colonies a tendency to decry welfare work for women and children. It has been said, and said in all seriousness, that it is useless to spend money on this work until the economic and general health conditions of the country have improved ; that a high infant mortality-rate strikes a balance between the population and the means of subsistence, and that it is better to let the children die if the world is not a fit place for them to live in.

At the first glance there may appear to be truth and reason in this argument ; but on further consideration it is seen to be a superficial observation and fallacious. In the first place, population and means of subsistence do not necessarily balance each other ; a low population may be and frequently is due to ignorance on the part of the mothers, even in a land of plenty. Secondly, it ignores the fact that welfare clinics are meant primarily for the education of mothers and not for the treatment of sick children. Thirdly, it fails to consider that, when no welfare work is done, many infants will die off, and many of those who survive will grow up in a weak and sickly state. The law of the survival of the fittest may hold true for the jungle, but among human beings its truth is less manifest, as the loving care and foresight of even ignorant parents will frequently interfere with its action.

I hold firmly to the belief that the State has a definite duty towards every child which it has allowed to be born within its boundaries, and that, in the words of the Declaration of Geneva, ' The child must be given the means requisite for its normal development.'

It is, of course, of little use to continue welfare work without at the same time improving bad economic conditions.

But, it will be asked, where can we get the money for all this ? The answer is that, while lack of money is undoubtedly one of the greatest handicaps to medical progress in the colonies to-day, yet a maladjustment in the expenditure of the available money is often an equally serious drawback.

Medical administrators have always before them the difficult problem of how to distribute the medical revenue in the most equitable way. It seems to me that they sometimes give insufficient attention to the value to be derived from

teaching the women of a country how to bring up a healthy family. Again, in the early development of a country it would seem necessary that a relatively large proportion of medical expenditure should be devoted to the training of local staff, of which an adequate number should be women.

II. THE PRESENT POSITION AS REGARDS THE HEALTH OF WOMEN AND CHILDREN

It is extremely difficult to make any general statement as regards health conditions which will be applicable to all colonies—the position varies too much from one colony to another. It can, however, truthfully be said that the standard of medical work in most of the colonies is high ; medical experts who from time to time pay visits to these countries often express their admiration at the excellent work being done.

In most colonies there are one or more first-class hospitals, staffed with highly skilled medical and nursing staffs, and frequently possessing the most up-to-date scientific equipment. There are several good medical schools, some excellent research institutes, and, in a number of the colonies, large public health works which can compare favourably with those at home, and there are well-qualified staffs of doctors and nurses actively engaged in improving health conditions.

Unfortunately, all branches of the work are not equally good ; as regards the medical work among women and children and the training of local nurses, the standard is not always high and seldom approaches that of work of this nature done at home. Once again, however, it is difficult to make a general statement. There are a few colonies where the infant and maternal mortality-rates are comparable with those of England ; there are some where the number of male and female patients receiving treatment at the hospitals are almost equal ; some where careful attention has been given to the training of local nurses, midwives and health visitors ; some where a number of women medical officers are actively engaged in gynaecological and general hospital work among women. Much of this progress is of recent growth and is evidence of an increased interest on the part of the medical authorities in the matter. Infant welfare work has been given particular attention, and in many colonies to-day very excellent child welfare work is being done.

On the other hand, in a few colonies the position may be considered definitely unsatisfactory, and in many colonies where some progress has taken place there are still certain aspects of the subject in which much improvement is required ; it is chiefly with these aspects that the present report deals.

There is, for example, frequently a grave disproportion between the number of male and female patients receiving treatment at the hospitals. There are districts, even those where most of the women are Mohammedan and, therefore, unwilling to employ men doctors, in which no woman medical officer is employed. The training of native nurses and midwives may be almost totally

neglected, even though the men of the country have been trained as doctors. In some places welfare work is almost entirely left to voluntary effort. Infant mortality figures of between 300 and 500 per 1,000 have been reported, and maternal mortality-rates as high as 30 or over are on record for certain districts.

It is difficult to give an accurate account of the position, as the returns given in many annual medical reports are meagre. Few colonies have yet been able to give accurate vital statistics for the whole area ; infant mortality figures are often given for certain districts, but not many colonies give figures for maternal mortality. In a number of colonies the number of male and female out-patients attending hospitals are given, but remarkably few give figures for male and female in-patients.

As an example of an unsatisfactory position we may take the following figures for native male and female patients for an African colony for the year 1934 :—

Native out-patients : { Male : 248,046 / Female : 79,414

The figures for European out-patients, however, for this same colony were :—

Male : 384
Female : 405

I think that it will be found that women generally require more medical attention than men, chiefly as the result of child-bearing, and that therefore the disproportion in the figures from some colonial hospitals is serious.

As another example I may quote from the annual medical report for 1934 for a colony which has been under British rule for about 40 years. As much as 12 per cent. of the total revenue of this colony is spent on medical work, and out of a population of about one and a half millions as many as half a million attendances were recorded at Government hospitals and dispensaries.

Yet what is the position as regards women and children ? There is practically no welfare or maternity work done by the Government, apart from that done in the general hospital routine ; and the figures for male and female patients at the hospitals are as follows :—

In-patients : { Male : 6,536 / Female : 2,111
Out-patients : { Male : 79,431 / Female : 35,375

In reference to this, it is instructive to read the words of the chief medical administrator, who in the annual medical report for 1934 writes as follows :—

' . . . the infantile mortality rate, according to such figures as are available, is a high one. Unfortunately the Medical Department is practically destitute of means of attacking this problem, as with the exception of the Native Hospital at Z——, where one European nursing sister is posted, no European women are employed by the Department on nursing or health duties in connection with the general African population.'

Again :—

' The difficulty of dealing with maternity cases at hospitals at which the staff often consists of a Medical Officer and male native attendants is a very real one. Every effort is made to attract native women to act as nurses, but from practical experience it would appear almost impossible to do so unless there is at the same time a European nursing sister employed at the hospital.

' Further, the provision of a European health sister at one or two of the larger hospitals would allow of the commencement being made with infant welfare and ante-natal work.

' Maternity wards already constructed could be opened and put to their proper use and native nurses could be trained and instructed in midwifery.'

Later we read :—

' Owing to the financial situation it was found by Government impossible to augment the staff of European sisters or to include provision for any health visitors and the two clinics (at X—— and Y——) already erected are being utilized to increase the ordinary hospital accommodation.'

It should be mentioned that these two clinics, together with the maternity wards, were built with money from the Colonial Development Fund—money which was given in order that this backward colony might advance medical welfare work among children and mothers.

To what factors can we attribute the neglect of the work among women and children in this colony ? To poverty ? But there are other colonies equally poor, yet in which conditions are better ; moreover, this country has had very considerable help given to it by the Colonial Development Fund. To the comparatively brief time it has been under British rule ? But in other colonies which have been in our care for a similar time the position is vastly different ; further, even in colonies which have been more recently taken over the position is frequently much better. To the backward character of the women of the country ? But here again investigation and comparison with other countries show that this alone cannot be held responsible.

Undoubtedly each of these factors plays a part in rendering such work difficult ; but after a comparison with the progress achieved in other countries I am convinced that the major causes which are responsible for the continuance of such backward conditions in several colonies are as follows :—

1. That there has often been and still is a lack of appreciation by the authorities of the great importance of this work.

2. That there is sometimes a lack of balance in the medical expenditure, i.e., there is a disproportionately small amount allotted to work among women and children, and that money and staff may be divided according to geographical areas rather than to the needs of different sections of the people.

The second factor is often dependent on the first, but it may also be dependent on the fact that a medical administrator, even though he appreciates the importance of this work, has his hands tied by the magnitude of the demands of existing organizations for curative work. He may be forced to continue this work in some cases by a particularly powerful or vocal section of the

population, and in others by the insistence of keen specialist officers on the fulfilling of their requirements.

It appears to be most important that a medical administrator should be free to balance his expenditure equitably among all classes of the community according to their needs, and that, where a special demand is made by any section over and above its share, then a special contribution should be demanded from that section.

Turning to another colony, we find in the 1934 medical report that in one town, out of a total of 23,886 in-patients treated in the Government General Hospital, only 6,024 were females, and in another town, out of a total of 9,024, only 1,536 were females. (In these towns there is a ratio of about 60 females to 100 males, but even allowing for this there is a marked disproportion.) The cause of this disproportion is not at first obvious ; the hospitals in both these towns are exceptionally well-equipped and staffed with skilled doctors and nurses ; there are good welfare centres and well-run maternity hospitals, which are popular with the women.

I have discussed this lack of popularity of medical institutions among women in certain colonies with many doctors and nurses, and the following causal factors for the position have been suggested to me :—

1. That the women cannot leave their homes unattended in order to enter hospitals. But their homes are unattended if they are seriously ill and bed-ridden, and they appear to enter maternity hospitals readily. Moreover, in other colonies where the position is better, it is equally difficult for women to leave their homes.

2. That they are frightened by the officialdom of the large modern hospitals, in which the friendly personal element is somewhat lacking, and that the rather alarming officials who act as porters are not sometimes above demanding money before allowing the women to enter.

It would appear that this second suggestion is important and that care is required in the selection of porters and other hospital extern officials, and continual watch needs to be kept on their conduct. The first complaint—that there is a less friendly atmosphere in large hospitals—may, to some extent, be overcome by choosing and appointing a special type of reception clerk. Certain older women, such as former nurses, midwives or health visitors well known in the district, might be appointed, who could act as a guide and adviser to the rather alarmed and puzzled women.

3. That in many colonies there are not nearly enough women medical officers employed in the hospitals. I am convinced that this is frequently an important cause. In many places where the difference in male and female patients in Government hospitals is marked, a mission hospital in the same area where women doctors are working may often be overcrowded with women patients. Experience in several colonies shows that, where a woman doctor of the proper type is employed, more especially for gynaecological and venereal

diseases work, the increase in the number of women patients may be remarkable.

4. That there is not enough welfare work being done among women, and that there is often a marked dissociation of welfare work from hospital work. This is an important point, to which careful attention should be given. Where such a dissociation exists, much of the time and money spent in welfare work is wasted, and the benefit to the community which should result from such work is greatly diminished.

The separation of these two forms of medical work has arisen from the mistaken policy of splitting medical work into two distinct branches—medical or curative, and health or preventive—sometimes with separate staffs, institutions and budgets. Such an artificial division has given rise to certain difficulties at home, but the results in the colonies can be much more serious. The policy now being recommended to colonial governments and increasingly being adopted by them is to unify the work as much as possible, and to give all medical work a preventive aspect in so far as this can be done.

I think that by attention to the above points improvement will be shown ; there may, however, be other causal factors for which careful study is required locally.

In some colonies lack of progress in the work among women and children may arise from a too strict adherence to methods of organization in force in other more highly developed countries, the attitude of the authorities being that these are the ' right ' methods, and that unless the work is done in the ' right ' way it should not be done at all.

They may consider that it is better to have a small piece of work done well in a limited area, and a few workers well-trained, rather than work indifferently done and workers of a less skilled type distributed widely throughout the country.

I formerly held this view myself, but further study of the subject, and, more especially, conversations with workers in India and Malaya, have convinced me that in many countries there is need for a combination of both methods, provided adequate and continual supervision is given to the less skilled work.

As an example of conditions to which the above remarks apply, I will give extracts from the medical report for 1934 from one of our colonies. In the section dealing with maternity and child welfare we read :—

' The direct activities of the Government have been limited to the training of midwives . . . and to the provision of a trained midwife at each of the rural hospitals. . . A still more disappointing feature is the apparent lack of Indian women of sufficiently good education to enable them to attain the modest standard laid down for candidates for midwifery scholarships, so that we are faced with this situation that the Indian community will not employ midwives who are not of their own race and are unable to produce suitable women of their own race for training. . . .

' Moreover, the opportunity has been lost. It will be seen in the following paragraph that the Midwives' Board decided to recommend to the Government the discontinuance of the training of midwives. . . Numbers of those women are unable to earn a livelihood in the profession in which they have been trained, and the effect of this departure on the part of the Government in its efforts to raise the standard of midwifery has been negligible.'

The seriousness of the position will be more apparent when we realize that the majority of the people in this colony are Indians—no less than 268,649 out of a total population of 393,328.

The density of the population may have had some influence on the authorities, and may perhaps have somewhat retarded their enthusiasm for maternity and child welfare work. For the whole colony there are 544·25 persons per square mile, and in one district the figures rise to 3,418·44. It is difficult to believe, however, that the best way to deal with this problem is to allow mothers and children to die ; some more constructive, more humane and more courageous policy than that is surely required.

The success in India of the system of training uneducated Indian women in simple and cleanly methods of midwifery, and of supervising their work by means of well-trained health visitors, convinces me that the hopeless attitude of the medical authorities towards the work among the Indian population in this colony is not justified.

Maternal mortality. Only a few colonies are at present able to give accurate figures for maternal mortality-rates. In some colonies the rates given are comparable to those in England. In some, though the rates appear high, they are in keeping with an equally high general death-rate. In a few colonies, however, the maternal mortality-rates are high in proportion to the general death-rate, and in some cases are even increasing yearly, though the general death-rate may be decreasing. Rates of 9, 12 or 15 per thousand live births are not uncommon, but in some parts the figures may rise to 20, 30 or even more.

The seriously high figures are generally reported among plantation estate labourers, and chiefly, though not entirely, among East Indian women. I have been assured by those who have studied the matter that the mortality is mainly due to an anaemia which becomes extreme if pregnancy supervenes. Much useful research work has been done into its causation.

There appear to be different types of anaemia among such women, macrocytic and microcytic types having been described, and these may have different causes. Three chief factors, however, have been incriminated : nutritional deficiency, hookworm, and malaria.

The first appears to be very important, and even when one of the last two factors may be causal the first may also be playing a part.

Whatever be the exact cause, death of mother and child can generally be prevented, provided the condition is recognized in time and adequate treatment given.

The main consideration, of course, should be to prevent the condition arising, and preventive measures, such as better estate sanitation, anthelminthic treatment and above all seeing that the women obtained an adequate diet, should be instituted.

It would sometimes appear that, whatever the regulations, practical difficulties may prevent the women from obtaining a good diet. Provision of land

for vegetable culture is of little use if the labourers have not leisure or sufficient energy after their work to cultivate it. Good wages will not help much if the marketing facilities are not adequate. Abundant and varied supplies of food will not avail if the women do not make use of it.

It would appear very necessary that all estates should employ a midwife or a health visitor, preferably of the women's own race, who would instruct the women in health matters, who would have special instruction in the premonitory symptoms of the disease, and who could produce suspicious cases to the doctor for adequate treatment.

Again, careful consideration of the regulations as regards maternity benefits seems to be required, in order that these should not be wasted but should be given at such a time and in such a form as to effect a definite improvement in the health of both mothers and children.

III. THE EDUCATION OF GIRLS AND WOMEN FROM A PUBLIC HEALTH POINT OF VIEW

One cannot consider the welfare of women and children without paying particular attention to the education of girls, women teachers and adult women, the former being largely dependent upon the latter.

A. The Education of Girls

It is being increasingly recognized that lack of girls' education is seriously retarding medical progress in the colonies. Medical funds have to be spent in educating women in clinics and in their homes, and this is mainly required because the education of the girls in the schools is deficient.

The development of work in hospitals, dispensaries and welfare centres is being made very difficult because it is impossible to get a sufficient number of educated native girls who can be trained to take part in the work there.

In an annual medical report for 1934 from an African colony, the Director of Medical Services writes :—

'Unfortunately among those of our candidates who have had an education such as might enable them to become genuinely efficient nurses there are as yet no women and the essential nursing problem is still to solve. The solution lies with the Department of Education.'

Again, in the report of the Pan-African Health Conference held in Johannesburg in 1935 under the auspices of the League of Nations, we read :

'A difficulty encountered in some countries is that African girls are, as a rule, insufficiently educated to permit of their being efficiently trained as nurses and midwives, although on the other hand, boys and men with sufficient education are available everywhere.'

And in a resolution passed at the end of the Conference it is asked :—

'that the Governments concerned will endeavour to further by every possible means the education of African girls.'

Serious social problems arise if education in a country is confined to boys. The inhabitants of several colonies have already complained about this, and have asked that the women of their country should be given more facilities for education.

A distinguished colonial administrator has written as follows :—

' Equal opportunities should be provided for the education of boys and girls. There can be no true development of a race unless the women as well as the men are educated. Among the African races the women are usually the guardians of native institutions. There can be no sublimation of primitive and often barbarous customs if the women remain uneducated. Nor can there be happy marriages and real home life, and all that this means to the children, if women are uneducated. It has been demonstrated in many areas in Africa that uneducated women are a brake on the wheel of progress. Experience in Asia as well as in Africa in the past forty years bears this out.'

On the same subject a celebrated colonial educationalist writes :—

' Girls' education has had too little attention paid to it . . . this is serious and much more serious than is generally realised. To begin with, it means a large number of uneducated homes in the next generation. For most educated men it will mean a dual life, one lived amongst male friends, where the considerations which govern thought and action are much like those of Europe ; another in the home, where fear of the unlucky and the unknown makes life to them irritating and irrational, and paralyses effort. Economically the education of a girl is worth more, I believe, than the education of a boy. It means, if the education is sensible, healthier homes and children, more intelligence in the community, more progress in character and in domestic and agricultural work within the next few years. The man, educated by himself alone, does not as a rule pass on much to his home or to his children, for in the home the obscurantist wife dominates the educated man, and educated men who cannot find anything but such wives become restless and discontented. They are apt to stand loose from their tribe and people. One-sided education in this way breaks up village and tribal life, and increases immorality.'

Much seems to have been written on the need for education for girls, but in some colonies little seems yet to have been done.

I think we can fairly ask for information as to who or what is responsible for the present unsatisfactory position.

Is it lack of money, or a badly balanced educational budget ? Is it a faulty educational policy, due to a lack of appreciation on the part of the educational authorities ? Is it that the type of education given to the girls is unpopular among the people ? Is a sufficiently definite policy being adopted towards guiding public opinion in this matter ? Is it that insufficient experimental schools are being developed in which to discover a popular and effective type of education ? Or is it that, while primitive people realize that most women spend a great part of their lives in keeping a home and bringing up a family, our educational policy in the colonies regards these occupations as though they were merely accidents which might or might not occur in the women's lives ?

Developments in girls' education in the colonies

It would be most unfair, however, to give the impression that no interest was being taken in this subject. In many colonies several different systems

have been developed which show promise of success, and thought is being given to the solution of this educational problem.

I have listened to much discussion and controversy on this subject from a variety of workers in many colonies. Some appear to think that education for girls is only advisable in so far as it can make them efficient home-keepers and mothers. The specialized health officer is sometimes accused of taking this point of view. I have heard a teacher complain that health officers appeared to think that the aim of the Education Department should be to turn out merely efficient reproductive machines.

Another body of opinion seems to think that the training of the mind and the teaching of an appreciation of ' culture ' should be the aim of the schools ; that girls should be given an education little different from that of boys ; and that infant care and housecraft should be taught elsewhere than in the school.

Health workers sometimes make the criticism that the type of education given to girls in some schools is useless as a preparation for their future life ; that, apart from a smattering of anatomy and physiology, the girls are given little training in health matters ; and that even where a domestic science course is given it too often consists in the teaching of fancy cooking and elaborate needlework ; more serious still, that the teachers themselves are frequently ignorant of the requirements in the home-life of the women.

There is possibly a certain amount of truth in all these criticisms ; the conditions vary so much in different schools and in different colonies. It may be that those whose concern is chiefly with the health of the people belittle the importance of the good general education which is necessary in order that they may take a responsible and definite position in the country and show an intelligent interest in the welfare of the community.

On the other hand, it may be that some educational authorities fail to realize the paramount importance to a woman of having a happy healthy home and family life. They may not realize the great necessity in more primitive countries, where the home life of the girls sometimes leaves much to be desired, for school training in household management, in mothercraft and in character formation.

In so far as I have seen, however, the educational departments appear to be trying to strike a balance between the above-mentioned extremes. In several girls' schools in the colonies which I have visited, good courses in domestic science and in child welfare have been introduced.

In some African colonies the authorities have introduced the Jeans' system of education, which appears to be particularly suited to village life in primitive societies. In this system the teacher and his wife are given practical instruction in healthy methods of living of a type suitable for villages. The wife is given practical training in child welfare. Their training finished, they are then sent out to live in a village where they are expected by precept and practice to be an example and help to the villagers.

Several persons have spoken to me enthusiastically of this system. For its success it is essential to have the co-operation of both the health and educational authorities.

In some colonies a very useful collaboration of medical and educational departments has been attained. Health visitors teach in the girls' school, giving lectures and demonstrations, while visits of the girls to welfare centres are arranged ; there they are given practical instruction in infant care. The teachers act with the medical authorities in keeping health records of the pupils and in developing healthy habits in school life. A good example of such co-operative work which I saw was in connection with the Kalutura Model Health Unit in Ceylon.

In Nigeria, in the Mary Slessor Memorial School, the United Free Church of Scotland Mission have formed a most interesting school for girls in connection with an orphanage for rescued twins. The following account has been given :—

> ' They aim at getting girls who are of marriageable age to train them for their future homes. In this respect their work has fully met with the approval of the people round, and young men frequently send their fiancées to the school for training. The girls live in huts which are very similar to, but a little better than, the village huts, six or eight to a hut. There is one still better hut, a model one, in which each older girl lives in turn when acting as housekeeper for the community. Then there is farm work, cooking, marketing, and looking after rescued twins, all done in native style but a little better. They learn the importance of cleanliness, of pure water, of doing things at the right time, but all in village conditions. Then there is dispensing . . . first aid and simple medicine. Talks are held on the care of little children, native customs are thoroughly discussed, and the girls can often practise their new knowledge on rescued twins. . . . The girls learn improved methods of oil and soap making. In the class rooms they are taught reading, writing, simple arithmetic, colloquial English, singing and drill.'

Suggestions for improvement

It is, of course, easy to criticize ; knowledge of the conditions and some practical experience as a medical teacher and inspector of schools both in India and Africa make me realize that there are many difficulties. From that experience, however, from advice given me by teachers and health visitors in the colonies, and from an understanding of the unhappy results of ignorance among the women of a country, I would venture to make the following suggestions.

1. That relatively greater attention and a relatively greater expenditure should be devoted to training native women teachers, to arranging satisfactory living conditions for them subsequently, to the careful supervision of their work, and to supplying opportunities for revision courses. Further, that in the teachers' training course more attention should be given to a really practical study of health problems and their solution.

2. That in all girls' schools in the colonies a considerable proportion of the time should be devoted to the teaching of domestic science and mothercraft in a very practical manner.

3. That attention should be paid to the formation of character, to developing a social conscience, and to giving the pupils an understanding of the important

part which women should take in developing the social welfare of the community.

4. That in primitive countries and in many rural areas in more advanced countries, a simpler and more popular form of education should be evolved, modelled on the lines of the Mary Slessor Memorial School. In this the girls should be trained primarily for their vocation as wives and mothers. To this training could be added gradually more literary work, as the schools become popular. The development of broadcasting throughout the colonies would add greatly to the possibilities of good rural schools of this type.

5. That more co-operation should be shown between the various departments, especially educational, medical, agricultural and administrative, both in devising and carrying out an educational policy for women as for men and suited to the countries' requirements.

Domestic science courses

As regards courses in domestic science, it seems that these can scarcely err by being too practical and that they should be incorporated in the school life, so as not to be considered as an 'extra' subject and consequently of lesser importance. The training should go beyond the teaching of sewing and cooking, and should include general household management and child welfare. The following suggestions may be given.

An actual house or compound might be built or hired either in, or in close proximity to, the school grounds. This house should be similar to those in which the majority of the girls will live in later life, though it is advisable to add a few improvements to it from time to time. Collaboration with the medical department is necessary in order that this model house and compound should be of a sanitary type, attention being given to the provision of adequate light and ventilation, a good type of flooring, good drainage, and a good type of latrine.

The domestic science course should consist in the planning and carrying out of the entire work of this house by the girls. Attention should be given to such things as maintaining the compound and latrine in a sanitary condition, to the prevention of mosquito- and other insect-breeding, to the disposal of household refuse, to the care and storage of food and water, to the care and cleaning of the kitchen utensils, and to the washing of kitchen cloths and dusters. Laundry, cooking, dietetics, marketing and catering should all be taught, the girls in turn accompanying the teachers to market. The meals should be chosen and the budget prepared by the girls for a family consisting of parents and of children of different ages, one of which should be an infant.

Domestic science courses are sometimes given in schoolroom or laboratory with foreign types of cooking apparatus; the subjects taught may be fancy needlework, sometimes of too fine a nature and damaging to the eyes of the pupils; such courses may have some aesthetic value, but they will have little effect in improving the health of the people.

For the mothercraft class I think that a visit on certain days by a health visitor

is advisable and that the course should be made as practical as possible. When mothercraft training is given it would seem that sometimes the type given tends to focus the attention of the individual too selfishly on her own concerns. For girls' schools, mothercraft training should not be narrowed down too much to the individual family life, but girls should be taught to regard all children as requiring care. Attention to this aspect of the subject will hasten the abolition of social conditions which are harmful to children, and will help to develop a public conscience with regard to the prevention of cruelty to children.

The importance of giving training in domestic science and mothercraft might be illustrated in another way. Thus, at an international educational conference I once heard an educated African delegate say: 'We want our girls to be educated, but when you educate them they will not marry us.'

This remark was not, I am afraid, taken very seriously, yet it was, possibly, one of the most serious and important observations made at that conference. It should lead us to give emphasis to two things: firstly, to see that in girls' schools the pupils are led to consider wifehood and motherhood as noble occupations; and secondly, that boys and young men should be given a higher idea of marriage and of the respect and consideration to be given to their wives.

One other point with regard to the education of girls remains. Matrons in hospitals frequently complain that they have great difficulty in getting local nurses to accept responsibility, to obey and enforce orders, and to show powers of initiative. These are serious defects, which render it difficult not only to train local nurses, but also to promote them to senior positions. I think that girls' schools could help very much in this matter if the school life were organized so as to develop individual responsibility for certain duties: if, for example, school societies were formed with an elected committee to organize and develop certain branches of school life; if older girls were in turn made to assume responsibility for some duties; and if debating societies were formed in which the value of strength of character, of powers of initiative and personal responsibility to others were discussed.

B. The Education of Women Teachers

In countries where educational work is little developed, relatively great attention and a relatively large expenditure are required for the training of the teachers, and in countries where a general education in health matters is deficient a relatively large amount of time should be devoted to a practical study of health problems and their solution.

The criticism that teachers themselves have little knowledge of health matters is less true to-day than it was a few years ago; but I think that perhaps the training given to them in this subject is not always as practical as it might be.

In Ceylon, at Mirigama, there is an excellent training-school for men teachers for rural areas, where very practical instruction is given in house construction, house-, compound- and village-sanitation, vegetable and fruit culture

and other matters. It is hoped to start a similar practical training-school for women teachers later.

School-teachers are now being encouraged by the educational authorities to interest themselves in the home life of their pupils, and, for the sake of efficiency in the education of girls, it is very important that they should do so.

The senior educational authorities in the colonies who are responsible for educational policies are usually men, and I think that most of them will agree with me that they often feel doubt as to what knowledge is required by the girls in order to equip them best for their adult life. When planning a curriculum for use in girls' schools, therefore, they may consult with the women teachers. But if the experience of the latter has been limited chiefly to school life, and if they are unmarried, they may not always fully appreciate the problems which arise in the lives of their pupils when they leave school and have to care for a home and family.

For these reasons I think it is essential that there should be a close association of teachers with the parents of the children in the home. Further, I think that benefit to the country will be derived from this association, and that, when colonial governments realize more fully the importance of a scientific study of social conditions, it will be from the teachers that much of the most useful information will be derived.

In England, the Royal Sanitary Institute holds an examination in school hygiene, for which it has issued regulations and a syllabus. This is meant for school-teachers and also for health visitors doing school work. The syllabus is interesting, dealing not only with hygiene and physiology, but also with sanitation of school buildings and with the hygiene of child-life and educational methods.

In the more advanced colonies it might prove useful to institute a similar examination, a syllabus suitable to the local conditions being drawn up by the educational and health authorities.

C. The Education of Adult Women

When education for girls is widespread, and when the school curriculum provides for adequate and practical teaching in health matters, the need for the education for older women will not be so necessary. As conditions are now, however, with a scarcity of girls' schools, and with much ignorance on the part of women, adult education is essential.

At present this instruction, more especially in the towns, is being given by women health visitors who are sometimes Europeans, but in many colonies, where the training of local health visitors has been developed, by local women also. These workers visit the women in their homes and give them advice, and this personal contact is of great importance in spreading a knowledge of health, but demands much time, labour and money ; very many workers would be required to deal with the vast numbers of women in rural areas. Some more economical method must, therefore, be sought to supplement it.

Broadcasting. The development of broadcasting is still in its infancy in the colonies ; as regards the education of women in the villages, it has great possibilities, but for its effective use women will have to be organized into societies, and demonstrations will need to be given to these societies illustrating the talks.

Women's societies. Organizations for women in the colonies are, I think, much required. In the original tribal life of many of the people in the colonies such societies existed and in them subjects relating to the welfare of women were considered. When detribalization takes place among such people, the position of women becomes often more degraded than formerly, and they tend to lose the interest and self-respect which a definite and recognized place in community life gave to them. It is well to remember, too, that in the original tribal societies girls were generally educated in their domestic duties, in the responsibilities of married life and in their duties to the community. Some of the information given to them may not always have been such as we may consider suitable, but at least these important subjects were not ignored, as has rather foolishly been done sometimes in the more modern schools for girls.

Several organizations for women are already in existence : in Fiji, for example, there are committees of the older, more responsible women in the villages, who look after the welfare of the children and the cleanliness of the village houses, and who bring cases of sickness to the attention of the doctor or health sister when they visit the villages. I understand that these committees work well, and I consider it useful to encourage the women themselves to take on part of the responsibility for the village welfare.

In the Far East, in China, India and Ceylon, where the great majority of the people live in rural areas, it has recently been realized that co-operative societies which deal, among other subjects, with health and education, are a possible means of spreading information widely among the people, and at the same time of getting the people themselves to take an active interest in their welfare. It is recognized that it is impossible to provide out of the existing revenues sufficient schools, teachers, doctors and health-workers throughout the country, and, moreover, it is felt that the participation of the people themselves in the work renders it more acceptable, more effective and more enduring, and that there is less danger of misunderstanding and discontent arising when new ideas and legislation are introduced.

The type of organization and the subjects dealt with in these co-operative movements vary. In Ting Hsien in China, Dr. Yen has developed a very complete form, in which health and the welfare of women and children are included.

In some cases voluntary women's societies have developed in connection with Health Units such as that in Ceylon at Kalutura.

In India, the Central Government has agreed to give grants to all local governments which can produce satisfactory plans for village improvements; in such plans as I saw, maternal and child welfare figured largely.

One of the earliest of these rural co-operative movements was started in Jugoslavia in 1921, and a very good account of it is given by M. Colombain in the *International Labour Review*, vol. 32, no. 1, July, 1933. Of the activities of the women's sections of the societies he writes :—

' Courses are organized for women and girls on general health questions, certain kinds of agricultural work (vegetable gardens, flower gardens, poultry keeping) and household questions. The practical work is concerned with the rearing of children, methods of keeping the house clean and healthy, the preparation of food in a rational way (in particular, the new kinds of vegetables as grown by the young people), and the improvement of methods of work (clothes, bedding, disinfecting, preserving vegetables and fruits for the winter, etc.), as with the juvenile sections, exhibitions and competitions are organized.

' As the juvenile sections take part in the sanitary improvements in the village, so the women's sections help to promote tidiness and cleanliness in the house, regular payment of subscriptions, and regular attendance at lectures and meetings. The village is divided into a number of groups of houses. Each group is entrusted to a responsible person who gives advice, encourages individual work, generally supervises and makes a report. The households most favourably reported receive as a reward utensils of which they are in need. For the competitions between sections the prizes are larger, but are also of practical and educational value, for instance an up-to-date loom becomes common property.

' Lastly, the womens' sections are responsible for organising fêtes and concerts and for creating a real social life.'

I think that such societies are full of promise and might profitably be developed in many of our colonies.

In many countries a knowledge of hygiene and sanitation is spread by means of Health Exhibitions, held generally during a ' Health Week.' Such exhibitions can be of much use to women if they are sufficiently accessible. An interesting account of a very excellent Infant Welfare and Public Health Exhibition held in Kampala, Uganda, is given in the annual report of the Medical Department of the Uganda Protectorate for 1934, and those who are organizing such exhibitions would, I feel sure, receive many suggestions from a study of this account.

The Indian Red Cross Society have also many useful posters and booklets dealing with health educational work and suitable for tropical countries ; those dealing with maternity and child welfare are particularly helpful.

IV. THE ORGANIZATION OF MEDICAL WORK FOR WOMEN AND CHILDREN

Methods of Organization

There are doubtless many ways of organizing medical work for women and children in the colonies which will show good results, and it is also doubtless true that the type of organization which suits one colony will not necessarily be equally suitable in another.

There are colonies where organizations much like those in England are proving quite satisfactory. There are colonies where it has been found necessary to make special arrangements and to provide special hospitals for women and children. I think, however, that in most colonies success can best be obtained

by not making work among women and children too separate a subject, but by incorporating this work as closely as possible with other medical activities. Thus it would appear advisable in the majority of colonies to have general hospitals, portions of which are devoted to work among women and children, rather than special hospitals for women or for children ; and to have maternity wards or blocks attached to the general hospital, rather than isolated maternity hospitals.

Again, I believe that for the success of the work, in the interests of economy and for the efficient training of local staff, welfare centres should be situated near main hospitals or dispensaries, and that certain members of the medical staff should be employed in the work of both institutions.

On this last point I do not expect to have the agreement of all specialist health officers, but I have reason to believe that there is a growing body of opinion among medical workers that this system makes for greater efficiency, provided that it is recognized that prevention and education are the main function of the welfare centres, and that the medical officers taking part in the work have the necessary preventive outlook.

As regards rural areas, what I have seen of composite health centres has convinced me that these can be very valuable, and that their effect will be of a more popular and permanent nature than that of isolated medical dispensaries with separate welfare centres.

The Organization of Hospitals in Order to Provide Effective Treatment for Women and Children

It has been shown above that special arrangements often require to be made in order to render the hospitals popular among women—such arrangements as the development of welfare work, the employment of women doctors, and attention to certain aspects of hospital administration.

The Organization of Maternity and Child Welfare Work

In the colonies, opinion as to the necessary activities and duties of welfare centres seems to vary considerably. In some centres the work may consist of little more than the provision of milk and clothing to necessitous children. In some, health sisters may be left to deal with the work alone, no visit of a medical officer being considered necessary. In some, women medical officers have been asked to run such clinics without the assistance of any trained health visitor.

In a number of colonies, however, the infant welfare work is now of a very high standard, and during my recent tour I saw many excellent welfare centres in Malaya, Ceylon and Palestine.

I have had many interesting discussions on the type of organization which was proving most valuable in the colonies, from which I give the following points :—

1. That one of the most important duties of welfare organizations in the colonies at the present time is to provide one or more model centres in which local health visitors may be given a thorough training to enable them to take part later in the work throughout the country.

2. That for efficiency it is essential to have close co-ordination of welfare and other medical work.

3. That, in order that these requirements should be fulfilled, it is necessary to have a medical organizer of maternity and child welfare work and a superintendent (health sister-tutor) of health visitors.

4. That, in order that maximum effect should result from maternal and child welfare work, constant research must be made in the welfare centre as to the nature and prevalence of defects and diseases, their causes, and the most effective means of prevention. Without such research and subsequent preventive action, there will come a stage at which the infant mortality rate will remain stationary.

The organizer of maternal and child welfare work. The organizer of maternal and child welfare work in the colonies should, I think, in most cases be a woman doctor, and she should have had special training and experience in the organizing of this branch of work. In some colonies, as I suggest in another section, she might also be the adviser on medical work for women and children.

Her duties would be to make a survey of the requirements and special problems of the country, both urban and rural ; to draw up a plan organizing the work so as to provide for thorough training of local women to take part in the work ; to arrange for extension of the work by this means in rural areas ; to ensure that the preventive and the educational work in the centres was linked up with that of the existing curative institution, and that it fitted into the general medical policy of the colony. She should not be merely an office worker, but should take part herself in the work at the welfare centre and, in small colonies at least, in gynaecological, maternity or venereal diseases work in the hospitals.

The superintendent of the health visitors' training-school is a very important officer of the welfare organization. She should be a fully qualified nurse, with training in midwifery and public health nursing, and should if possible also have training and practice in the work of a sister-tutor.

In the smaller colonies, she might, I think, with advantage be the head of the combined training-school for nurses, midwives and health visitors. She should be responsible for the general work of the model welfare centre and for its subsidiary branches.

For the success of the work throughout the country, continual careful supervision of the rural centres and of the work of the health visitors must be made. This supervision can most profitably be shared between the organizer and the superintendent.

Welfare centres. The work in the centres consists generally of weighing the children, keeping records, examining the children and giving advice to the mothers as to requisite care of themselves and of their children. This work is generally very well done in colonies where trained workers are in charge, but a few remarks may be made.

In some cases the advice given and the methods recommended may be more suitable for conditions in England than in the colony concerned ; more originality is sometimes required as regards certain problems. Again, one cannot help feeling that a more simple form of record-keeping would sometimes be of benefit in the work, as a large proportion of the time of a health visitor is often now taken up in this work.

In some centres which I visited in India and in Hong Kong, a few cots were reserved for the use during the day of certain types of cases, such as children who required special feeding. The children stayed there during the day and the mothers were taught how to prepare and give the required food ; this would seem worthy of trial elsewhere. Another arrangement which is common in most Indian centres is to have facilities for the mothers to bathe their babies at the centres ; in crowded towns with bad housing accommodation this may be a boon to poor mothers.

In some colonies a very useful association of welfare organization with voluntary societies, or with workers engaged in social service, has been arranged with much benefit to the welfare work. Such an interest taken in the women at the clinics may be the means of adding greatly to their pleasure and education, and it also seems to make them accept the advice given more readily and encourages them to attend more frequently.

In some centres in Malaya I saw good educational exhibits, such as exhibits of suitable local diet, wax models of foodstuffs being shown and posters provided. In one centre demonstrations were given of methods of cooking food suitable for children, and of preparing substitutes for milk, such as soya-bean milk.

In some places, where a particularly large centre had been built in a central town, arrangements had been made to bring the mothers to the clinics by special 'buses. Opinion was divided as to the advisability of this ; and I am inclined to think that the better plan would be to train more local workers and to open more village subsidiary centres which could be visited from time to time by the staff of the central institution.

One of the most interesting activities in connection with welfare centres which I saw on my tour in Malaya and India was the training and supervising of the village type of uneducated midwife. I have been assured by those engaged in this work that the results have proved satisfactory, provided that care is taken in the selection of the women to be trained, that very simple and practical methods of training are given, and that careful supervision is arranged.

It is unfortunately true that in the villages the women will very often be unwilling to employ young midwives, even when these are well educated and

trained. They prefer the older women, even though they are unskilled. The above system appears to be successful in overcoming this temporary difficulty.

The provisions for maternity work. The arrangements for this work in the colonies must vary very much according to the ideas and customs of the people, to the housing accommodation, and to the ease with which access to homes of the people may be obtained.

Three chief methods may be employed :—

1. Special maternity hospitals or maternity wards in or attached to the general hospital. (I prefer the latter rather than separate hospitals, partly for economy, but chiefly because many ante-natal and post-natal cases require gynaecological or general treatment, and it is often difficult for uneducated women to understand why they should attend at another hospital for such treatment.)

The popularity of hospital treatment of maternity cases in many of the colonies is becoming yearly more evident, and there are now in many colonies very good hospitals for this work. They are especially popular in large towns where the housing accommodation is bad ; indeed, in several their popularity is already embarrassing the authorities.

There are, however, certain drawbacks to such hospitals. Firstly, they are expensive, and unless adequate fees can be paid may become a heavy drain upon the medical exchequer. Secondly, it may be difficult for the women to reach them in time. Thirdly, there is always the risk of spread of epidemics. Fourthly, they are not popular in countries where child-birth is surrounded by superstition and the performance of tribal rites.

The provision of a number of hospital beds for maternity work, however, will always be necessary both for complicated midwifery cases and for a sufficient number of normal cases to provide training for pupil-midwives.

2. Smaller maternity centres or homes are often useful if distributed in different parts of large towns and in villages.

Such small centres can be of great use. They are generally easily accessible for urgent cases, they are less terrifying for the women in many parts, and are, therefore, more popular. They need not be expensive to build, and in some cases rented houses can be suitably equipped. They can act as a central office and as a residence for the district midwives and/or the supervising health visitor, and the building may also be built and arranged to serve as a welfare centre.

It would appear to be most essential, however, that such small homes should be given strict supervision and that no attempt should be made in them to deal with major complications. I have seen some very dangerous conditions in maternity homes which were being run by voluntary societies who evidently did not realize the importance of supplying adequate supervision.

3. The provision of adequate numbers of well-trained midwives to attend to the women in their homes.

In many countries, even in towns this form of maternity service will be most appreciated, and it will always be required in the rural areas.

V. THE NEED FOR MEDICAL WOMEN

It has been found in England that there is always a certain number of women who prefer to go to women doctors for general medical advice and treatment rather than to men, and that there is a still larger number of women who prefer to go to them for certain complaints, more especially those of a gynaecological nature.

Moreover, in ante-natal, infant welfare clinics, clinics for women suffering from venereal diseases, and in the medical inspection of school-girls, women doctors are increasingly being employed.

The general standard of the work done by women doctors is now high ; there are hospitals entirely staffed by women, and each year adds to the valuable medical research being undertaken by women.

It seems necessary to draw attention to these facts, because it would appear that some colonial governments do not yet realize the very real need there is for the services of medical women, and the high standard of the work they can perform. In the colonies, the need for such workers is often very much greater than in England, especially in colonies where the local women are afraid of entering hospitals staffed by men, or where it is against their custom, as among Mohammedan peoples.

In India the definite need for women doctors has been recognized for many years. There is a separate Women's Medical Service for India, and very many hospitals are staffed entirely by women. There are five medical schools for training women doctors alone, in addition to general schools open to both sexes. There are now very great numbers of qualified Indian medical women distributed throughout India and working among their own people most successfully.

The position of the colonies, however, is remarkably different. It is true that in some colonies, such as Malaya and Hong Kong, we find a comparatively large number of women doctors; but in many of the African colonies there are none. In the whole of the East African colonies the governments employ only two or three women medical officers ; in West Africa there are eight women medical officers, six of whom are in one colony, while in another, with a population of about 20 millions, many of whom are Mohammedans, there are only two. In the majority of African colonies, therefore, no women doctors are employed, and yet it is in these colonies that there is generally an unsatisfactory disproportion between the number of men and women patients in hospital and that there are high rates of infant mortality.

Many missionary societies appear to be more alive to the needs of the people in this respect, and employ a comparatively large number of women doctors in their African stations.

It is surprising that this serious state of affairs continues year after year, and we may well wonder who or what is responsible.

In the colonies the creation of a post for a women medical officer lies to a great extent in the hands of the Director of Medical Services ; and, we may ask, why then should there not be more posts created ?

Firstly, because many medical administrators view the requirements of their colonies from a geographical point of view, rather than from the point of view of the needs of the different sections of the people. Secondly, because some of them do not yet appreciate the seriousness of the position, nor understand what valuable work a woman doctor of the right type and with the requisite training can do. Thirdly, I have been reluctantly forced to the conclusion that in a few cases the lack of women medical officers arises because of the personal prejudice against them on the part of the chief medical officer. It is difficult in these days to realize why this should be so, and it would appear to be very unfortunate that such individuals should be entrusted with the welfare of women and children in large areas of our colonial empire. Fourthly, because the medical administrator genuinely thinks that the value to the country of a woman's work is not equal to that of a man, and, where funds are limited, he has to consider the value of his expenditure carefully.

I believe that he is wrong in this opinion for two reasons. Firstly, he fails to consider that the medical work for women is much behind that for the men. Secondly, he does not realize what valuable work a woman doctor can do with proper organization of her work.

In several colonies where women have been appointed the scope of their work has been so narrowed and the area over which their work extends so restricted as to render them indeed rather an expensive luxury. Women doctors who go to the colonies to work among women and children should not, I think, be considered, nor should they themselves consider, that they are merely specialists in one branch of work : they should be competent and willing to take part in any work for women and children, and this should be explained to them and the fact should be dwelt on when selecting recruits for the colonial service.

I think the most effective and economical use of their services may be by arranging for them to take part in a number of associated activities such as some of the following :—

1. In organizing and supervising welfare work for women and children throughout the country.

2. In taking part in those branches of hospital work which can often best be done by a woman doctor, e.g., gynaecological work, clinics for women suffering from venereal diseases, and ante-natal and maternity work.

3. In medical inspection in girls' schools.

4. In acting as tutor and adviser to women students in medical schools.

5. In making a scientific study of the medical welfare of women and children, viewing this not from the view of the specialist, but widely and with an eye to future development.

In some of the more advanced colonies it may be possible and advisable to employ women as specialists, but for the majority of the colonies it is, I think, by sharing in the work of the above activities that the best result will be obtained from their services.

Medical administrators with many claims upon their scanty funds are entitled to demand a high standard of work from their women medical officers, and I feel sure that with careful selection of recruits, and by providing satisfactory conditions for the practice of their skill, the results will be satisfactory.

As the majority of women doctors going to the colonies are required for work among women and children, it is obviously essential that they should have thorough training in these branches of medical work; this generally presents little difficulty. The training for the organization of maternity and child welfare work, however, is not always so easily obtained; the course for the Diploma in Public Health gives only a small amount of time to this subject, and women who have had practical experience in such work are generally in senior positions and unlikely to vacate them for work in the colonies.

In India this deficiency has been rectified by the provision of a special course and examination for the Diploma in Maternity and Child Welfare given by the All-India Institute of Hygiene and Public Health.

Most of the women medical officers whom I have met in the colonies have shown a deep feeling of responsibility for the welfare of the local inhabitants. I believe that this feeling can be developed by encouraging officers to study and to make suggestions with regard to local problems, rather than by merely appointing them to fill this post or that, and to send in returns of the number of patients attended.

It would appear useful if, in the colonies, there were to be appointed special Advisers on medical work for women and children. I think that such a post should be filled by a woman, partly because I feel that a woman, provided she be of the right type, with adequate experience, will be most successful in it, and partly because it gives the women in the Service a chance of promotion to a position of responsibility, and so would encourage a good type of medical woman to take part in this work.

The duties of an Adviser would be to consider the medical requirements of the women and children in the colony, to find out and report how far these requirements were being met, and to suggest means for improvement. She would have to organize maternal and child welfare work, to see that adequate co-ordination of all branches of the work was effected, and to supervise the work of the other women medical officers in the colonies.

In small colonies she would generally have to take part in some form of the medical work herself. She would act as an adviser to the Director of Medical Services on all subjects relating to medical work among women and children.

I consider that a woman with the necessary personality and with adequate

experience would prove invaluable to the medical administrator. Much of her work would deal with conditions of work of the various types of women staff in medical service, and much of it would deal with social problems which affect the health of women. These are subjects in which the proper type of woman could give much helpful advice, and some of this work could only be performed adequately by a woman in many colonies.

The Training of Native Women as Medical Assistants and Doctors

In many of the colonies, especially in the smaller and poorer ones, the cost of a European medical officer's services are a serious claim on the finances. Such officers are, therefore, usually employed in central main hospitals and institutions and in supervising work in the districts, much of the routine medical treatment being performed by locally trained medical assistants.

Unfortunately, in many colonies little or no consideration has been given up to now to the training of local women as medical assistants. Thus, when a European woman doctor is appointed much of her time may be occupied in routine treatment, a considerable amount of which does not require a highly skilled officer. This is a wasteful method, and it is important that more attention and encouragement should be given to the training of well-educated girls in local medical schools.

In certain colonies, where medical schools exist, a few local women have already been trained, but in some, none at all, our policy in this matter showing a marked contrast to that of India. Of those already trained in the colonies, I understand that some have proved successful. I have talked with several of these women ; a few of them complained that they felt somewhat lost in the training-school, while some who had taken post-graduate courses in a woman's medical college in England considered that to be the most valuable part of their training, because there they first realized what scope there was for medieal women, and were able to develop confidence in themselves.

I think that success depends very much on this last point. In the special women's medical colleges in India the personalities of the students develop in a remarkable way, and they generally become capable and self-reliant doctors. In mixed colleges there is a tendency for them sometimes to become submerged in the much larger number of men students and to lack confidence in themselves. I think that care should be taken to prevent this, and that encouragement should be given them, and I suggest, therefore, that where there is a woman doctor available in a colony she should take some part in the work of the medical school and should if possible act as a tutor and adviser to the women students.

Knowing the success of training medical women in India, it has been surprising to me to find how little has been done in the colonies, and still more surprising that doubt should be expressed as to whether the local women were capable of being trained.

VI. THE TRAINING AND CONDITIONS OF EMPLOYMENT OF NURSES, MIDWIVES AND HEALTH VISITORS

For the improvement in the well-being of women and children in the colonies it is essential that there should be an adequate supply of well-trained local nurses, midwives and health visitors (public health nurses), and that these should be widely distributed throughout the country.

The training of nurses. The provision of well-trained nurses, and more especially the distribution of these throughout the country, presents many difficulties—difficulties much greater than those met with in most European countries. This fact must be acknowledged ; but at the same time it must be recognized that the difficulties are not insurmountable, and such difficulties should not be accepted as an excuse for inaction in the matter. They may, however, necessitate alterations in methods of training from those adopted in other countries ; they may also necessitate some originality in devising satisfactory arrangements for nurses' work subsequent to training.

Difficulties arise from many factors : from the secluded position in which the women in some colonies live ; from the lack of education among girls ; from the fact that girls marry very early, often on leaving school ; from the fact that conditions in the homes of the girls are very different from those in hospital, and that in many cases their up-bringing has not accustomed them to discipline, to ideas of service to others, or to the importance of self-control and personal responsibility.

Further factors are that attendance on the sick or on a maternity case may, among some peoples, be looked upon as unclean, and the profession of a nurse or midwife therefore as an inferior one. It is felt also that the work of a nurse is hard, off-duty time is scanty, the pay relatively poor, and the conditions of service subsequent to training often far from attractive.

This is a formidable list of difficulties and requires very careful consideration in planning methods for developing local nursing services.

I have talked with many different persons, doctors, nurses, teachers and parents, and there seems to be a consensus of opinion in many colonies that improvement in the training of local nurses is urgently required. I have been particularly struck by the almost universal feeling among nursing sisters in the colonies that improvement is needed both in the system of training local nurses and in the conditions of service for them once they have been trained.

Position in the colonies to-day. As in other matters in the colonies, the position in this respect is not uniformly bad.

For example, in Uganda the C.M.S. Mission has had a good training-school for midwives for many years and has recently started one for nurses. In Trinidad, I understand, there are some very good locally trained nurses, and recently a few of these passed the examination for health visitors of the Royal Sanitary Institute. In a few colonies sister-tutors have been appointed and good nurses' homes built.

In some quite small hospitals, where the sisters can give individual attention to the students, some very satisfactory nurses have been trained ; on the other hand, in some large, well-equipped hospitals, where results should be good, the nurses are given a very poor training because there is no special sister-tutor appointed to devote her time to training the nurses, and all the existing sisters' time is fully occupied in the many duties of hospital nursing administration.

In several colonies little or no attention has been given to the subject, the authorities evidently considering the position hopeless owing to the local conditions. In some colonies, though the native men have been trained as doctors, yet there are few native female nurses, the nursing being done by European sisters and male native nurses. In some, where there are numbers of educated girls, but where insufficient attention has been given to the requirements of training and of the work, a serious situation has arisen ; in such colonies the nursing profession has fallen into disrepute, respectable parents are reluctant to allow their daughters to train locally, and teachers are loth to advise their pupils to take up this work. In such colonies, for example, young girls who have recently left school may be admitted to the hospital wards without any preliminary training ; there may be no nurses' home, the girls being required to sleep out, there may be no sister-tutor to act as their guide and adviser, to develop their character and to give them pride in their profession.

Training of nurses in other countries. The training of nurses has been greatly improved in recent years, and it might be useful to give a brief account of certain developments which have taken place in several countries.

In England and Wales, for example, the position is as follows :—

There is a General Nursing Council appointed under the Nurses' Registration Act, which, among other duties, keeps a Register of Nurses, makes rules as regards the training of nurses and as regards the conduct of examinations for admission to the register. The prescribed training must be taken in such institutions as are approved by the Council. The Council has drawn up regulations and a syllabus of training for the guidance of training centres, planned over a three-year course ; some of these might be quoted, as they are equally applicable to the colonies.

' Ward management and hospital economy should have more care bestowed upon them. . . .

' District Nursing, Public Sanitation and Public Health are subjects of growing importance in the training of a nurse both to widen her outlook and to counteract the limited vision sometimes acquired in the sick wards. They should be brought to her notice from the outset, and kept in full view throughout her training. . . .

' The Council also attaches great importance to the giving of revision classes to pupils, the correction of their notebooks and the personal supervision of the pupil nurse in all stages of her training and study.'

As regards the training in approved hospitals in England : Candidates are

VI. THE TRAINING AND CONDITIONS OF EMPLOYMENT OF NURSES, MIDWIVES AND HEALTH VISITORS

For the improvement in the well-being of women and children in the colonies it is essential that there should be an adequate supply of well-trained local nurses, midwives and health visitors (public health nurses), and that these should be widely distributed throughout the country.

The training of nurses. The provision of well-trained nurses, and more especially the distribution of these throughout the country, presents many difficulties—difficulties much greater than those met with in most European countries. This fact must be acknowledged ; but at the same time it must be recognized that the difficulties are not insurmountable, and such difficulties should not be accepted as an excuse for inaction in the matter. They may, however, necessitate alterations in methods of training from those adopted in other countries ; they may also necessitate some originality in devising satisfactory arrangements for nurses' work subsequent to training.

Difficulties arise from many factors : from the secluded position in which the women in some colonies live ; from the lack of education among girls ; from the fact that girls marry very early, often on leaving school ; from the fact that conditions in the homes of the girls are very different from those in hospital, and that in many cases their up-bringing has not accustomed them to discipline, to ideas of service to others, or to the importance of self-control and personal responsibility.

Further factors are that attendance on the sick or on a maternity case may, among some peoples, be looked upon as unclean, and the profession of a nurse or midwife therefore as an inferior one. It is felt also that the work of a nurse is hard, off-duty time is scanty, the pay relatively poor, and the conditions of service subsequent to training often far from attractive.

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As regards the training in approved hospitals in England : Candidates are

required to pass a medical examination of fitness, to show a comparatively high standard of education, such as school-leaving certificate or matriculation. Age of admission normally varies from 18 to 30, but may even be up to 40. An eight-hour day is aimed at, students live in well-equipped nurses' homes with separate bedrooms, and facilities for recreation are provided.

Premiums for admission are now seldom demanded, salaries have increased, free board, laundry and uniform are provided, and pension or superannuation funds have been introduced. There is generally a trial period of from two to four months before a nurse is definitely accepted for training in hospital.

One of the most important innovations in hospitals in England recently has been the organizing of preliminary training-schools in connection with the various hospitals, in which the students are given a theoretical course of lectures with practical instruction in certain subjects before entering the hospital wards. This course lasts for from 6 to 12 weeks in the different hospitals. Special sisters—sister-tutors—devote their entire time to the training of the nurses.

As regards subsequent training, the College of Nursing in London, through its education department, gives a special course of training for nurses wishing to become hospital administrators and sister-tutors, and several other institutions do likewise. Courses of lectures are also given for the Diploma in Nursing of the University of London.

Certain hospitals in America and Canada are staffed by permanent trained nurses, student nurses being given their training in them much as medical students are given theirs, i.e., by a theoretical course of lectures and by systematic practical work in laboratories, wards, and out-patient clinics under qualified medical and nursing instructors. Again, in some hospitals arrangements are made with universities, and degrees in nursing are given, part of the course being taken in the university and part in the hospital.

In the Peiping Union Medical College in China both of the above systems are followed. Students pay a fee on admission and also fees for the first year's training, after which tuition is free. This College also offers to qualified nurses two courses in institutional nursing : one, covering a period of eight months, preparing students for special positions in teaching and administration in hospitals ; and one, covering a period of five months, preparing students for supervisory and head nurse positions.

In the Central School of Nursing, Nanking, an interesting system has been developed. The purpose of this school is to prepare a group of nurses of a general type who will be fitted to work in the field of curative medicine, public health and midwifery in both urban and rural districts. The course of study, exclusive of a probationary period of six months, covers a period of three years (two years' hospital nursing, six months' public health nursing, six months' maternity and child health).

The hospital in which the training is given is staffed by a permanent staff of trained nurses.

TABLE I

Giving a summary of systems of training in several English hospitals

Hospital	No. of beds	No. of sister-tutors	No. of home-sisters	No. of probationer nurses	Time spent in preliminary training-school	Duration of trial period	Duration of training	Age on admission
Guy's Hospital	647	2	4	219	16 weeks	8 weeks	3 years + trial period	19–40
London Hospital	839	1	9	305	7 weeks	7 weeks	3 years + trial period	19
St. Thomas's Hospital, London	593	4	2	80–90	9 weeks	8 weeks	3–4 years	21
University College Hospital, London	540	3	3	180	10 weeks	10 weeks	3 years + trial period	19–30
Royal Infirmary, Liverpool	336	2	2	84	8 weeks	16 weeks	4 years	19–30
Royal Infirmary, Manchester	750	2	3	198	12 weeks	12 weeks	3 years + trial period	21–30
Royal Infirmary, Bristol	412	2	3	140	7 weeks	12 weeks	3 years + trial period	19–30

It will be noticed from the above examples that nurses' training has developed from the mere apprenticeship in hospital wards of former days to a systematic planned education; that special tutors are appointed to guide and supervise the pupils; and that early courses of instruction are given in the preliminary training-schools.

Suggestions for training in the colonies. In the colonies the above provisions are even more important, since the poor education which many of the girls will have received both at home and in school will require to be considerably supplemented in the training-school. Moreover, for the same reasons, careful individual

attention by special sisters is required to develop character, and encouragement and advice must continually be given.

It would therefore seem essential that in every colony, whether wealthy or poor, *there should be a preliminary training-school for nurses and a special sister-tutor should be employed.* A nurses' home with a motherly type of home-sister or housekeeper is, I consider, also essential.

I would suggest, too, that the period to be spent in the preliminary training-school might, where possible, in the colonies be increased to six months. Pupils who are obviously unfitted can be eliminated at an early stage, and, provided the course of training is wisely planned and includes the study of personal and domestic hygiene, the knowledge obtained will be equally useful in after-life for girls who do not complete the full nurse's training. When other hospitals are approved for the training of nurses, an arrangement might be made by which student-nurses took their six months' preliminary training in the central school and went later to the different hospitals for their practical work in wards.

The personality of the sister-tutor is obviously most important (possibly even of more importance than her scientific knowledge, as much of the teaching will also be done by sisters in the hospitals), and this fact should be considered in recruiting such women. It is she who will chiefly be responsible for the tone of the nursing profession in the colony. Sister-tutors need not necessarily be recruited at home—sisters already in the colony who show special aptitude for teaching and a sympathetic interest in the nurses may usefully be appointed for the work.

The syllabus for the preliminary course in the training-school will require very careful thought in order that it shall fill the local requirements.

When education is deficient in a colony, more tuition will be required in such subjects as the keeping of records and of temperature charts ; where living conditions are primitive, explanation will have to be made of hospital equipment, such as laid-on water, electric light and other electric apparatus ; where the home-life of the girls fails to develop self-control and reliability, more attention to character training will be required.

As regards the development of character, of making the girls self-reliant, resourceful and reliable, very definite methods of training towards this aim must be considered. Vague preaching on the subject is useless.

In a training-school for midwives in Senegal I once saw an example of such training. I was shown essays which the pupils had written week by week on such subjects as the following :—' How I showed initiative in my work this week,' ; ' An example of how I kept my temper under provocation ' ; ' An example of how I tried to uphold the dignity of the nursing profession.' The essays were discussed by the pupils together later. It will be noted that these essays were not merely repetitions of lectures which had been given to the pupils, but required original thought on their part.

Matrons of hospitals often complain that local nurses are incapable of

accepting responsibility and are therefore unable to be promoted to senior posts. They often lay the blame on the type of education given in the schools. To a certain extent the schools may be at fault ; but I think much education in taking responsibility, in developing self-control in emergencies, and reliability in routine duties can be given in the nurses' training-schools. Pupils may be given increasingly important duties for which they are individually responsible, and given gradually more important supervising posts. Debates by the pupils in which the importance of these points are discussed would prove effective.

In the colonies subjects such as anatomy and physiology may need simplification, as, I believe, in many training-schools too much detailed instruction is at present given in these subjects. More attention will need to be given to diet and to practical teaching of invalid cookery suited to local conditions and prejudices.

Again, much more time needs to be allotted to the study of personal and domestic hygiene and to the prevention of disease.

We have a great opportunity in the colonies to-day of planning an improvement in the training of nurses. At home, the methods adopted for nurses' training were often formed when nurses' work was regarded solely as caring for the sick. Attempts are now being made to give student-nurses a preventive outlook also ; but in older institutions this may take some time to develop. In many of the colonies, on the other hand, we have more opportunity to do this, since medical organizations are still in the process of development.

In training nurses there, I think that we should aim at giving them all a preventive outlook, and should plan the syllabus accordingly. We should so arrange that nurses, midwives and health visitors can all in their subsequent work be exponents of healthy methods of living.

I am suggesting the following scheme for training with this aim in view, and with the idea also of providing more suitably trained nurses as health visitors. The need for health visitors will, I feel sure, extend greatly in the next few years ; they will be required in out-patient departments of hospitals and in dispensaries, as well as in welfare centres and schools, and will be engaged increasingly in other public health activities.

As regards the training of hospital nurses, the scheme varies little from that recommended by the General Nursing Council for England and Wales, but a certain amount of alteration as regards the sequence of the subjects taught would be required, so that those of a purely institutional value would be relegated to the last year in so far as possible. Where hospitals depend very greatly upon student-nurses for the nursing of patients, this may prove an almost insurmountable difficulty. I agree with those, however, who think that such dependence upon student-nurses is neither good for the patients nor for the nurses, though I recognize that economic considerations at present may make it necessary in some hospitals.

Where this difficulty does not arise, and where the authorities are anxious

to train a large number of health visitors with the necessary preventive outlook, I think that this scheme might at least be given a trial. At the same time, I recognize that some authorities consider that it is an advantage for health visitors to have had a complete three years' training in general nursing.

Suggestions for Training Nurses in the Colonies

At least three kinds of nurses are required : hospital nurses, midwifery nurses (midwives) and health visitors (public health nurses)*. They all require the same basic training in the causes and prevention of disease, in the requirements of the human body, and in the care and advice necessary when the body is diseased.

In some colonies such well-trained nurses are all that may be required ; but in many others it will be found impossible as yet to obtain a sufficient number of educated girls to train, and in many it will be found very difficult for these young women to work in rural areas alone, or to become popular and readily accepted by the older, uneducated women. In such colonies I think we must decide *as a temporary expedient* to train a lower type (Grade B) of nurses of all three kinds.

These nurses would be chiefly uneducated women who would be trained in the town or village in which they lived and would be given very simple and practical instruction in duties which require less skill.

Basic training for all Grade A nurses. I think that all nurses could take the same course for, say, two or two and a half years, with specialization in their respective subjects later. This might be arranged as follows :—

Preliminary training in training-school	6 months.
General training in hospital	18 months.
Midwifery in hospital and district	6 months.

The training of the Grade A midwifery nurses ends at this stage.

After this the hospital nurses and public health nurses would specialize in their respective subjects.

Hospital Nurses

Grade A. Those nurses who have completed the basic training and decide to continue working in hospitals can now be given a special training in such subjects as ward-management, theatre work and the supervision of junior nurses and domestic staff. Special care needs to be taken to ensure that these nurses will be capable of being promoted to senior positions, such as staff nurses and ward sisters ; at present, complaints have frequently been made that this has not been possible ; to a certain extent, I think, the training has been at fault.

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*The title ' health visitor ' is used in this report because it is most commonly in use in the colonies. The title ' public health nurse ' is used in some, as in Ceylon. I think it is preferable.

Grade B nurses would require very definite training, and a limitation should be put upon the duties they should perform.

Midwives

Grade A. The opinion as to the duties of and training required by midwives varies surprisingly throughout the world. By some a six months' course in midwifery is considered enough, while by others a complete three years' nursing training plus six months' midwifery is considered essential.

*In England there is a Central Midwives' Board which administers the requirements of the Midwives' Act, regulates the training of midwives, and supervises and restricts their practices within due limits. It also issues and cancels certificates, holds examinations, and keeps a Midwives' Roll. Candidates for examination must have received training in an approved institution.

Two types of midwives are recognized : (1) nurses who have had general training for three years, followed by six months' midwifery ; (2) women without the above general nursing training, but with twelve months' training in midwifery.

The rules for training are interesting and should be carefully studied by medical authorities in the colonies. One point is particularly worthy of note : of the twenty patients which the student must personally deliver, the first five must be attended within an institution where there is training approved by the Board, and, of the remaining fifteen, *at least five must be attended in their own homes.*

In the colonies I think we are missing an opportunity if we do not arrange for midwives to have at least some instruction in hygiene as well as in child welfare, for they spend much time in the homes of the people and have many chances of giving advice. I think, therefore, that we should aim at giving midwives a training such as the basic training recommended above, wherever this is possible. Moreover, these midwifery nurses would prove of great use in small hospitals in rural areas, where, if there is no midwifery work to engage their attention, they could assist in hospital or dispensary work.

Grade B. In the majority of the colonies, however, it would be impossible at present to obtain enough educated women to train as Grade A midwifery nurses, and it may therefore be necessary also to train a Grade B type as a temporary expedient.

In many countries the necessity of different types of midwives has been recognized. In India, where caste and custom restricts practice very much, it has been found necessary to select, from among the older, uneducated women who formerly practised midwifery, certain promising members, and to give them a thorough training in simple cleanly methods of midwifery ; to provide them with midwifery bags with the necessary contents ; to train them to

*At the present time Parliament is considering legislation which aims at improving the training and practice of midwives.

recognize certain complications which require more skilled attention, and to send for this in time. A register is kept of these midwives, and they are continually and carefully supervised by trained health visitors, to whom they report each case which they attend.

This system has also been adopted in parts of Malaya, and I have been assured by many persons competent to judge in both these countries that these women often prove successful, provided careful selection is made in the first place and continuous and careful supervision provided later.

In some colonies another type of short-trained midwife has been produced. Girls and young women have been chosen and sent in from the villages, or have volunteered and have entered hospital and taken a short course of training—in some cases of six months' duration, in some of nine. These pupils are generally of poor education ; many of them have come from primitive village homes ; they are not accustomed to hospital equipments and to such things as laid-on water and electric light, and much of their first month at least must be spent in getting accustomed to their novel surroundings.

I have taken particular trouble to enquire of the authorities of the success of this type of midwife. In many cases, I was told, it was unsatisfactory, the time spent in training was too short, in most cases no sister-tutor was provided to give special attention to the pupils, and above all there was generally no provision for training in the homes of the people, as the hospitals where the training was being given had no service for domiciliary midwifery. This last is a very serious drawback. Sometimes these poorly trained midwives were turned out to practise midwifery without adequate supervision being arranged ; no arrangement had been made for revision courses. The results have in some cases been disastrous. Thus, I have heard a Medical Officer of Health deploring the high maternal mortality in his town, and attributing it in most cases to puerperal sepsis which often occurred in cases attended by these inadequately trained midwives. Moreover, in some towns where many of this type of midwife had been trained there was considerable unemployment among them ; many of them were young and had not been able to gain the confidence of the people.

When such short-trained midwives are considered necessary in a colony, I would suggest that the course should certainly not be less than one year ; that a large part of the training should be given in district work ; and that continuous and careful supervision of their work should be carried out.

Health Visitors (Public Health Nurses)

Grade A. The duties of health visitors are chiefly of an educational nature, advising women both in clinics and in their homes on health matters ; the need for their services therefore varies inversely with the education in health matters of the women of the country.

In the colonies where education is deficient and where women often live secluded lives, the employment of health visitors is very necessary ; home

visiting may be the only means of associating them with medical work and of popularizing western ideas as regards prevention and cure of diseases.

European health visitors who are engaged for work in the colonies are usually required to have passed the health visitors' examination of the Royal Sanitary Institute. The Institute has not so far a special tropical health visitors' examination ; with the increasing employment of health visitors in the tropics, however, such an examination might well be considered.

On appointment to the colonies, some of these health visitors have been expected almost without assistance to develop welfare work and to train local health visitors. In some districts, where there has been an unfortunate lack of co-operation between medical and health departments, no arrangements have been made for visits of a medical officer to the welfare clinics. The reasons sometimes given for this omission are that there is no need for such visits, that welfare centres are only meant for prevention, and that the presence of a medical officer would turn them into dispensaries.

I consider such a point of view most short-sighted, and can only think that the holders of it do not realize that doctors also can and should have a preventive outlook.

In a few centres which I visited run on the above method, I found the educational work being done very well ; but some of the health sisters in charge told me that they often had difficulty in persuading the women to go to hospital for treatment when required ; they also stated that they frequently felt doubt as to the best advice to give and as to the significance of symptoms, and for these reasons would have been relieved to have had the advantage of periodical visits of a medical officer. They found it difficult to find time to train local health visitors, more especially as regards giving them theoretical lectures.

Since extension of welfare work throughout the colonies is so very dependent upon a supply of well-trained local health visitors, it is important to give careful consideration to the training they should have and to the way in which it should be given.

In most colonies at present the local health visitors are required to have three years' hospital training, six months' midwifery training and at least six months' health visitors' training. Most of those with whom I discussed this training considered that a full three years' hospital training was not necessary, and in many cases was a disadvantage, as it tended unduly to magnify the importance of curative work.

In India, health visitors are not required to have previous hospital training, but only midwifery training ; this is followed by twelve to eighteen months in the health visitors' training-school. I think that a certain amount of hospital training is an advantage, and the basic course recommended above for all types of nurses should, in my opinion, give health visitors a very good preparation for their special study.

The lectures for the health visitors' course can be given in the nurses'

training-school and the practical work in the model welfare centre. Where possible, it is advised that training should also be given in a less well-equipped centre in a poor part of the town and supplemented by visits to nearby village centres.

As regards the syllabus, most of the subjects taught for the Royal Sanitary Institute health visitors' examination would appear equally suitable for the colonies, with modifications for local conditions. More attention to diet, to social and domestic economy, to prevention of insect-borne disease, to house- and compound-sanitation, and to the storage of food and water are required. The prevention of certain diseases, such as malaria, and the many diet-deficiency diseases require special instruction.

Again, when the Grade B type of midwives is employed in a colony, the health visitors must understand how to train and supervise their work, and, if Grade B health visitors are employed, how to co-operate in a useful manner with them also.

Grade B. Doubt has been expressed by most of those whose advice I have sought as to the advisability of training a lower-grade health visitor. It has been pointed out to me that health visitors' work is chiefly educational, and could not therefore be performed by uneducated women. I still think, however, that they may be necessary for a few colonies ; for example, where it is impossible for male sanitary inspectors to enter the homes and compounds of the people, owing to religious objections, and where there are not enough educated women for Grade A health visitors, or where a section of the people are totally uneducated and will not permit visitors of another race to work among them. Such Grade B health visitors would act as a liaison between welfare doctors or Grade A health visitors and the people, gradually educating the latter to welcome more skilled workers. They should be recruited from the people among whom they are to work, and a limited amount of duties should be required of them.

The Provision of Satisfactory Conditions for Nurses Subsequent to Training

If nurses, midwives and health visitors are to be enabled to do satisfactory work throughout a colony, careful arrangements must be made as to where and how they are to live.

Certain safeguards are necessary if the nursing profession is to be considered a respectable one, in which the better type of girl will wish to work. Safeguards are particularly necessary where the women hold an inferior or markedly dependent place in the community, and where the position of a single woman living by herself may give rise to misunderstanding.

I have consulted with many people as to the most satisfactory measures to take, and may summarize the advice received as follows :—

Firstly—and I think most important—a thoroughly good training must be

TABLE II

Scheme for training nurses

Basic training for all types of Grade A nurse

(Total period 2½ years)

	Nature of study	Where given	Duration of course
1	Preliminary course (emphasis on domestic and personal hygiene and on the prevention of disease)	Chiefly in the nurses' training-school	6 months
2	General training in care of the sick (emphasis on the patient, less on ward technique)	Chiefly in hospital wards and out-patient clinics	18 months
3	Midwifery training (emphasis on the care of patients in their own homes)	In maternity wards and in district work	6 months

Subsequent special training for Grade A hospital nurses

Nature of study	Where given	Duration of course
Training in ward administration, theatre work, surgical nursing, supervision of junior nurses and lower-grade nurses	Hospital wards, theatre, nursing administrative offices and stores	1 year and over

Subsequent special training for Grade A health visitors

Nature of study	Where given	Duration of course
Training in child hygiene, health visiting, and other public health activities (emphasis on lectures on dietetics, social and domestic economies, also in training and supervising lower-grade midwives and health visitors and in domestic sanitation)	In lecture-rooms of nurses' training-school In welfare centres (model and more primitive types) In houses of people	1 year

given to the girls, so that they understand fully the requirements of the situation and appreciate how much their own character and conduct matter.

Secondly, arrangements should be made for continual supervision of girls placed in smaller towns and villages, by means of visiting sisters or by women doctors.

Thirdly, wherever possible younger nurses should live in a hospital where there is a European sister or older responsible local nurse in charge. This naturally applies to nurses required for hospital work, but it applies also to nurses, such as health visitors, whose work lies outside hospitals.

Fourthly, senior women of the district should be encouraged to take a personal interest in the welfare of the nurses. It may be convenient to form a society for this purpose in a colony, with local branches in the districts. Government officials and other influential local persons should be asked to see that respect is given to the girls, and should themselves guide public opinion in this matter.

Fifthly, nurses should be encouraged to form their own nursing societies and to hold meetings among themselves from time to time.

Sixthly, revision courses, which are necessary for continual efficiency and keenness, help also to make the nurses feel that they are not neglected ; they should be arranged for where possible, and attendance at them should be required before giving promotion.

Seventhly, it may be possible for nurses to be placed in districts in pairs, or to share quarters with the women teachers.

Lastly, in certain places it may be advisable to employ only married women or older widows, more especially in outlying villages or towns.

I see no adequate reason for the hopeless attitude sometimes adopted by medical authorities towards the development of a good service of local nurses ; but it must be recognized that a definite, well-thought-out policy, suitable to the local conditions, is essential for success.

The normal requirements then may be summed up as follows : thorough training, careful supervision, and satisfactory arrangements for nurses in outlying districts. In colonies, however, where education is deficient, a temporary arrangement is necessary, by which two grades of nurses may be trained, i.e., a well-educated grade for the highly skilled work, and a less educated grade for simpler duties.

One last remark is necessary with reference to the important subject of the discipline of the nurses. In all cases, I think nurses should be instructed to look to the senior woman in immediate charge of them, whether matron, sister-tutor, health visitor, or visiting sister, for redress of their grievances, and, except in the case of major offences, disciplinary action should be entrusted to these senior women.

VII. SOCIAL CONDITIONS AND THE WELFARE OF WOMEN AND CHILDREN

In talking to those engaged in medical work in the colonies, one cannot help being impressed by the constancy with which a great deal of the ill-health among the people is considered to be attributable to ignorance, to poverty and to harmful social environment and customs.

The attention of the doctor or nurse must therefore be continually concentrated on these factors if improvement is to be brought about.

Poverty. The causes and effects of poverty were considerations formerly considered to lie somewhat outside the duty of a medical officer. In recent years, however, the medical profession has been insistent on the necessity of good housing, adequate sanitation and a pure water-supply, and have conducted careful scientific investigations into the food requirements of the people. In the colonies also, the medical authorities in many places have been active in investigating local deficiencies and requirements. They have been persistent in advocating improvement, and, in several colonies, such as Kenya, in educating the people on the means by which they can themselves improve their economic position.

The health of women and, even more, the health of small children depends very much on economic conditions, and much of the sickness seen at welfare clinics, both among mothers and children, can be attributed solely to poverty. I have heard a health sister beg to be excused from visiting among a particularly poor section of a people, because she felt it was ironical to talk of a balanced diet to people who had practically nothing to eat but rice, or to advocate cleanliness when water and fuel were only obtainable with great difficulty and soap was a rare luxury. Yet in that country there were extremes of wealth among the local communities. One wonders if colonial governments are active enough in trying to render conditions more equal. Is this indeed beyond the capabilities of the local financial authorities ?

I think it is impossible to continue to ignore the fact that children in some colonies are dying from malnutrition, and that mothers are sometimes forced to discard their new-born babies. Courageous thinking and planning for a policy of economic improvement and for the better equalization of wealth is necessary. If this is impossible, then we must face the advocacy of birth-control ; yet in a colonial empire of two million square miles with a population of only fifty millions, this seems not only a feeble but a dangerous policy to adopt.

Harmful social customs. There are in many colonies social customs or practices which have serious consequences on the welfare of women and children. For example, there is the belief that it is necessary to kill twins, and children whose upper teeth appear first ; there are dangerous rites associated with new-born infants and with recently confined mothers ; and there are harmful practices, such as that of female circumcision.

It would clearly be unwise for governments to introduce drastic legislation

for the suppression of these practices until the people understood why such legislation was being enacted ; but is public opinion now being sufficiently educated in these matters ? I think that where such practices are known to be causing misery and ill-health, the local government should have a clear policy on how the people should best be educated so that public opinion would be arrayed against such practices.

Certain social problems. There are very many social problems which require investigation in the colonies, and I can only touch on a few of them here.

Why, for example, should mothers discard their children, to die of exposure, or sometimes to be eaten by dogs ? Has adequate investigation been made of the cause, and have the necessary remedies been considered ?

There are colonies where children are adopted in large numbers ; do adequate safeguards always exist for their welfare ?

There are colonies where 76 per cent. of the children are illegitimate ; have the fundamental reasons for this been discovered ? What effect has this on the welfare of the children ? Are the single mothers able to support both themselves and the children ? Are well-run crêches supplied for young children whose mothers must work ? Would better housing accommodation improve this position ?

In rapidly growing towns, is sufficient free ground being reserved for playgrounds for children ? Are there in many colonies societies for the prevention of cruelty to children ?

Again, when male labourers have been withdrawn from villages for labour elsewhere, have the effects on the women and children in the villages from which they go been sufficiently studied ?

In a number of towns of recent growth in Africa, many of the people who have come to reside there have become detribalized. Has adequate study yet been made of what social consequences these changing conditions are having upon the women and children ?

Where women hold a very low position in the community, and where the education of girls continues to be neglected year after year, have we thought out any clear policy as to how public opinion could be altered in favour of advance ?

These are a few of the problems which I have encountered and to which my attention has been drawn by others interested in the welfare of women and children.

I cannot deal with them more fully here because I have not had sufficient opportunity to study them ; those to whom I have spoken about them have complained that frequently no accurate study had yet been made, nor was there, in some cases, any person competent to study them satisfactorily.

I would suggest that in each colony there should be formed some committee or organization whose concern would be the welfare of women and children. The need for such an organization is greater in more primitive

societies. Such an organization does not necessarily imply any official increase in staff, but rather a definite policy with regard to collecting appropriate information by those most capable of obtaining it, and of having this information put before the government, who can then consider it and take what action is necessary and wise. Missionaries, anthropologists, women medical officers, nursing sisters and health visitors can supply much information, as can also teachers who visit in the homes of their pupils, wives of government officials, if they interest themselves in social conditions, police officials and others. The information required will generally come, and indeed it is best that it should come, in the daily routine work of such persons ; but the facts need to be recorded, and information requires to be pooled and its significance studied.

In some places, and especially in towns or districts where rapid changes or upheavals in social conditions have taken place, specially trained social workers might be appointed to make a careful scientific study of the circumstances.

SUMMARY AND RECOMMENDATIONS

1. In a considerable number of the colonies, the welfare of women, looked at both from a medical and an educational point of view, has made less progress than that of the men. Serious situations have arisen in some colonies as a result of this inequality, and it is advocated that an energetic policy towards improvement should be adopted. It is recognized that there are often important difficulties to be overcome, but it is considered that these are not insurmountable, provided appropriate and energetic measures are taken to deal with them.

2. Improvement in the health of the people in many colonies is being delayed because of the lack of education for girls. Many more girls' schools are required, and in some cases more original methods of education would appear to be necessary.

3. An expansion of the teaching of hygiene, domestic science and mothercraft is required in schools, and also in the training-schools for women teachers ; such training should be given in a really practical manner.

4. More thought might be given to devising methods by which local women may be educated to take a larger share in the promotion of social welfare in the community ; such methods as the formation of women's societies or women's branches of co-operative societies are suggested.

5. Increased attention to welfare work and to the instruction of women in health matters is advocated, as well as increased facilities for and, in some cases, more popular forms of hospital treatment for women and children.

6. A vigilant watch needs to be kept on the rates of maternal mortality, more especially among estate labour.

7. In a large number of the colonies much more determined efforts and better-planned systems appear to be needed for the development of local services of well-trained nurses, midwives and health visitors. It is suggested that the

method of having two grades of these workers, which has proved successful in some colonies, might usefully be extended to others.

8. Special preliminary training-courses for nurses and the increased employment of sister-tutors are strongly recommended.

9. An increase in the number of medical women is advocated, more especially for Mohammedan countries. It is suggested that in some colonies senior women medical officers might act as advisers on the medical work for women and children.

10. Increased opportunity in certain colonies is advocated for the training of native girls to become medical assistants and doctors ; arrangements for women tutors to guide them in their studies are considered necessary.

11. There are certain adverse social conditions which are proving harmful to women and children in several colonies. Sufficient attention does not always appear to be given to these at present, and it is suggested that definite organizations to study social conditions might be formed in the colonies.

Conditions vary so much in the different colonies that the above remarks are not equally applicable to all ; but since the subject has been treated in this report without reference to particular colonies it has been necessary to make somewhat general recommendations.

ACKNOWLEDGMENTS. I am greatly indebted to the Trustees of the Leverhulme Research Foundation for the grant of a Fellowship for the purpose of this investigation, and also to the Liverpool School of Tropical Medicine for granting me leave of absence from duty.

My thanks are also due to many government officers, and more especially to the officers of the medical and nursing services, in all the countries visited, for their very helpful advice and willing assistance. It would have been a great pleasure to me if space had permitted my mentioning individually all those who, amidst their many duties, found time to explain their local problems and the methods adopted at present to deal with them, and who gave me their suggestions for improvement.

Finally, I should like to have had space to mention all those kindly people of diverse races who showed me so much hospitality, and who made my tour such a pleasant and interesting experience.

MISCELLANEA

A SIMPLE AND RAPID METHOD FOR EXTRACTION OF THE SALIVARY DUCTS OF TSETSE-FLY, SUITABLE FOR TRYPANOSOMIASIS EXAMINATIONS

The chloroformed fly is held in the hand, and the legs are snipped off with the scissors. A fine pin is then inserted through the pleuron of the thorax at an angle of roughly 45°. By means of this pin the fly is fixed and completely immersed, ventral side uppermost, in normal saline contained in a small dissecting-dish, having a layer of paraffin wax at the base, and of dimensions suitable for placing on the microscope stage. A second pin is similarly inserted through the pleuron of the other side of the thorax, so that the fly is firmly held in position on the wax. The abdomen of the fly is now snipped off, in the saline, with fine scissors.

A slide having been placed in readiness, with a drop of saline on it, the fly is then examined under the dissecting microscope. If the palps still adhere to the proboscis, it is as well to separate them from it with a needle. The proboscis is then gripped with fine forceps along its length, right down to the bulb expansion at its base, and a gentle and steady pull is given until it is removed. Whilst the pull is being exerted, the object is observed under the microscope, and great care is taken that the attachments of the proboscis give way gently and steadily, and not with a sudden jerk, the force exerted with the forceps being regulated accordingly.

The proboscis is then completely removed and mounted on the slide, and the salivary ducts will be found still attached to it and ready for examination.

This simple modification of Lloyd and Johnson's method, and also of the modification of the latter submitted by Corson in 1933, at any rate as applicable to the material of *Glossina morsitans*, on which it was tried, seems to have the advantage not only in the possibility of a higher percentage of duct extractions, but also in the fact that any necessity of observing or handling the fine threads of the ducts themselves during the operation is obviated. Only the relatively large and hard object—the proboscis—has to be dealt with during the manipulation, the ducts being allowed entirely to take care of themselves and being invariably extracted with the proboscis, as subsequent examination under the microscope shows.

ERIC BURTT.

24 *February*, 1936.

RELAPSES IN CUTANEOUS LEISHMANIASIS*

BY

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(*Received for publication* 8 *June*, 1936)

The cutaneous lesions of leishmaniasis manifest themselves in an eruptive form with a single centre, to which they usually remain localized. The centre may vary from a minute papule to a large bulla (in one of my cases the lesion was 13 cm. in diameter). The eruption may remain unchanged in size for a long period ; it may ulcerate, in which case healing proceeds from the base upwards and from the periphery to the centre, or it may not ulcerate but be slowly absorbed. The lesion resembles that of an ordinary furuncle, but without its acute and severe manifestation ; some writers use the term ' leishmania furunculosis.'

There is, apparently, a protective reaction round the nest of parasites, sufficient to confine the disease to a limited area. In some cases this tissue-reaction is insufficient, and there is an invasion of neighbouring lymphatic glands in which L.-D. bodies are found. Moreover, in some cases, new secondary lesions appear along the course of lymphatic channels. This is clearly illustrated by the following case :—

A child, 18 months old, was brought to the clinic on March 13th, 1934, with 15 boils on the face, 12 of which were ulcerated and covered with haemorrhagic crusts. The lesions had already lasted for 6 months. The L.V.R.† (leishmania vaccine reaction) was positive. Along the course of a swollen lymph vessel, leading from one boil to the enlarged submental lymph-glands, were indolent nodules covered with a shining bluish-red skin. These nodules were only a few weeks old (see Pl. IX, fig. 1).

Histological examination revealed tubercles consisting of epithelioid cells, and in some lymphatic vessels elements resembling the remains of parasites were found. The nodules found in the course of the lymphatic channels must be considered as bubonuli leishmaniae.

In large lesions of long standing, I have sometimes noticed at the periphery of the infiltrated area minute papular elevations, which persisted for a considerable time. They did not develop, and later vanished with the complete disappearance of the initial lesion.

These minute papular elevations are probably metastatic growths in the

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*A lecture delivered at the Eleventh Dermatological Congress held in Budapest from September 13th to September 21st, 1935.

†The L.V.R. is a test which is used for diagnosis of leishmania. (Cf. Dostrovsky, 1935.)

skin caused by the parasites on their passage from the original lesion through the lymph-channels.

A special and unique form of leishmania is reported in the following cases :—

Case 1. M.K., a Palestinian girl of 17, gave a history of being infected with leishmania of the skin in 1924. There were 3 lesions on the forearm and the legs. She was given an injection of neosalvarsan 0·15, and did not continue the treatment as her condition had improved. This case was described in a previous paper (Dostrovsky, 1925). In 1926 the patient returned to the hospital for treatment of another disease. Small tubercles of a brownish-red colour were found round the periphery of the old lesion. According to the patient the tubercles appeared in clusters round the scar after the original sore had healed (see Pl. IX, fig. 2). Mantoux, Wassermann and luetin tests were negative. No leishmania parasites were found. L.V.R. test was positive. The histological examination by Professor Getzova on April 22nd, 1926, revealed the following :—

In the corium there was an area of necrosis containing a number of elongated seeds (epithelioid cells ?). The small areas of infiltration contained many polymorphic cells (L.-D. bodies ?) and an accentuated parakeratosis. The anatomical diagnosis was leishmania (?) of the skin. The patient refused further treatment.

In April, 1927, the patient returned for observation. The clinical picture at that time showed that the circinate eruption was more prominent and somewhat larger than when first observed in 1924. The smear taken on April 3rd, 1927, was positive for leishmania. Two pieces of tissue were excised (for histological examination), one from an old lesion and the other from a recent one at the periphery of the scar.

Histological examination by Professor Getzova revealed the following :—

Specimen 1 exhibited two definite well-defined nodules; the more superficial nodule, covered with thin epidermis, consisted of several smaller nodules formed of epithelioid cells which were rich in protoplasma. In the smaller nodule giant cells of Langerhans were observed. The anatomical diagnosis was leishmania of the skin.

Specimen 2. The tissue in various places was composed of large cells, but not giant ones. Intracellular inclusion bodies were found (disintegrated parasites ?).

The patient refused further treatment. She next appeared for observation on April 22nd, 1930, when her condition was found unchanged. Wassermann, Mantoux and luetin tests were negative; L.V.R. positive. Parasites were not found. The clinical diagnosis was leishmania recidiva orbicularis. The patient received X-ray treatment but did not return for further observation.

Interpretation of the case : A young asthenic girl, very emaciated, appeared at the clinic 6 years after infection with leishmania, and showed leishmania of nodular form, the nodules having a circinate arrangement around the old

leishmania scar. During the whole period of observation leishmania parasites were found only once. The treatment was occasional and inadequate. The histological examination confirmed the diagnosis of leishmania.

CASE 2. A Jewish-Persian girl, 7 years old, stated that at the age of 2 years she suffered from a boil on each cheek. (In Persia these boils are called 'sachma'.) After treatment with domestic remedies the lesions healed, but parts of them persisted at the periphery of the old ulcer. Every summer these lesions increased in size and in the winter became smaller.

Examination disclosed that the young, poorly nourished girl had on her cheeks two irregular scars with well-defined edges. These were somewhat deep and less rich in pigment than the normal skin. At the periphery of the scars there was a brownish-red infiltration, consisting of non-ulcerating tubercles with a serpiginous arrangement. On palpation these were somewhat hard. When pressed with a slide, the infiltrated region appeared a yellowish-brown, but there were no elements resembling sago to be seen. They were painless and did not cause any subjective complaints on the part of the patient. Wassermann and Pirquet tests were both negative. The L.V.R. was positive. No parasites were found. The patient objected to the taking of a specimen from the lesion for histological examination. The clinical diagnosis was leishmania recidiva tubero-serpiginosa.

After receiving 5 injections of leishmania vaccine, the patient left the clinic, and did not return until August 7th, 1932. On both cheeks, at the periphery of the sunken scars, there were the same foci that existed formerly. The serpiginous character of the lesions was more accentuated. The colour was yellow The foci were covered with small scales. The intradermal tuberculin test (1 : 40,000) was negative ; the L.V.R. was positive (3 cm. in diameter even after a week). Physical and X-ray examination of the chest revealed no abnormalities.

The histological examination of a specimen of the lesions revealed a number of foci resembling nodes with epithelioid cells, many giant cells of Langerhans and irregularly scattered lymphocytes. No necrosis was observed. Tubercle bacilli were not found. Anatomical diagnosis : tuberculosis (lupus form ?) or a tuberculoid form of leishmania of the skin (?). Animal test for tuberculosis was negative. The clinical diagnosis was leishmania recidiva tubero-serpiginosa.

Interpretation of the case : A young girl of about 10 years of age, asthenic in type, presented a serpiginous lesion of the papulo-tuberculous type, slowly advancing from the periphery of old scars of leishmania on both cheeks, without any tendency towards healing. The L.V.R. was very much accentuated. Diagnosis : leishmania recidiva tubero-serpiginosa.

CASE 3. A Jewish-Iraquian girl, of about seven, came to the clinic on April 15th, 1929, complaining of a boil of about 3 months' duration on her right cheek. The centre was in the process of healing, but at the periphery, especially at its lateral border, there was still an active process, i.e., an area of infiltration, brownish-red in colour, painless and non-ulcerating. Smears taken from

4–5-year-old scars on the nose and on the left cheek showed leishmania parasites. About the right angle of the mouth was a lesion still in the process of healing.

The patient did not again visit the clinic until December 15th, 1929. At this date an annular lesion was noticed on the right cheek, the colour of which was reddish-brown, with a border covered with crusts. The histological specimen revealed no parasites at the site of incision, but the histological picture suggested leishmania. The patient was given a small dose of X-ray radiation. She did not finish the prescribed course of treatment.

When she returned on November 27th, 1930, only an active and outwardly progressing lesion (diameter 4·5 cm.) on the right cheek could be seen (see Pl. IX, fig. 4). The shape of the eruption was roughly that of an arch, made up of a series of semicircular bands (about 3 mm. thick) of small yellowish to dark-brown papules covered with scales. Three foci showed the usual leishmania scar. Microscopic examination showed no leishmania parasites, though the L.V.R. was much accentuated. The Wassermann and Pirquet tests were negative.

The histological examination of a specimen taken on December 2nd, 1930, from the active part of the eruption disclosed foci of epithelioid cells, polymorphic nuclei in the corium, several giant cells of Langerhans and indurated epidermis in a state of slight hyperkeratization. The surface of the papules was flat. Leishmania parasites were not found. The anatomical diagnosis was most probably leishmania of the tuberculoid form. The clinical diagnosis was leishmania recidiva. Treatment with CO_2 resulted in considerable improvement. The boil had sunk, but there was no complete healing. The treatment was discontinued by the patient.

In the following August the patient returned for further observation. At this time the lesion showed no further advance of the active process. Smears showed no parasites. Culture for leishmania (by Professor Adler) was positive. Treatment with intramuscular injection of Fouadin and application of 3 per cent. tartaric acid was given. Complete recovery took place.

Interpretation of the case : A young girl of asthenic type, very emaciated, was suffering from cutaneous leishmaniasis which had not been treated systematically and in which the eruptive process was progressing slowly at the periphery of the lesion. The appearance was that of a poly-semicircular, spreading, micro-tuberoid lesion resembling lupus ; it disappeared only after 6 or 7 years. Parasites were not detected directly, but the culture was positive.

There were three additional cases ; but the history of the cases mentioned above makes it unnecessary to go into further detail of the development of the disease, and it will suffice to discuss the salient points characterizing the three additional cases.

In one of these, a girl, 8 years old, suffering from leishmania, came to the clinic a year after the boil had healed. (In the picture the scar was brought out very clearly ; see Pl. IX, fig. 5.) At the same time minute tubercles

appeared on the healthy skin at the periphery of the scar and at some distance from it. Clinical diagnosis : leishmania recidiva nodosa.

The other two cases were similar.

DISCUSSION

The outstanding feature of these cases was the chronicity of the process. Case 1, for example, was under observation for a period of 6 years. While the skin reaction was a very slow one, associated with a healing process centrally, there was a gradual spread peripherally, where there developed brownish-red tubercles covered with scales, non-ulcerating and indolent, arranged round the scar either in the form of a complete circle, as in the first case, or simply in groups of tubercles near the scar, as in cases of the second group, or in the form of multiple semicircular narrow bands of tubercle on one side of the scar, as in Cases 2 and 3.

In *differential diagnosis* tuberculosis, in the form of lupus, and tertiary syphilis must be considered. And the possibility that one of these conditions may have gained a foothold in a region where there was very little local resistance must be taken into account. (This is in accordance with the well-known fact that a tattooed area is often liable to be affected by a syphilitic eruption.) But the frequent negative tuberculin reaction tests, the negative animal tests, the absence of lupoma by epidiascope examination, the negative Wassermann and luetin tests, all exclude tuberculosis or lues. On the other hand, the connection with the leishmania scar, the clinical appearance, and the positive leishmania culture, as in Case 3, prove that we are dealing with a special form of dermal leishmania which we may call leishmania recidiva.

The pathogenicity of recurrent leishmania of the skin can be understood from the introductory note to this paper, where observations were made on the minute nodules developing as metastatic forms at the periphery of the larger boils. Usually these daughter nodules do not reach their full development, but are absorbed with the resolution of the central focus.

In the cases described above, the centre of the lesion healed first, while the nest of parasites remained at the periphery.

The following are some of the specific reasons for the recurrence of the leishmania eruptions. The degree of immunity developed in the body was inadequate for the destruction of the parasite, but sufficient to minimize its virulence. Thus the poly-semicircular progression originates as a response to an incomplete immunity. On the one hand, we see a localized immunity causing healing with scar formation, and, on the other hand, the formation of new foci by the parasites. (Because of this, the intradermal positive allergic reaction of the vaccine was particularly interesting.)

It was, however, very difficult to decide the reason for this deviation from the usual in leishmania lesions. The following factors might have been the cause.

All the patients were *young, asthenic and poorly nourished.* It is possible that these factors determined the type of reaction. We must also consider the inadequate and unsystematic treatment as an additional cause. The treatment disturbed the immunity reaction, and, at the same time, did not exert sufficient destructive action upon the parasites—as in Case 1, where the patient received only one small dose of neosalvarsan, and as in Cases 2 and 3, who received very inadequate X-ray treatment. I believe that one or more of the above-mentioned factors (or some similar factor diminishing immunity) is responsible for the production of this type of leishmania recidiva which shows close resemblance to the forms of tertiary lues and the lupus form of tuberculosis.

SUMMARY

1. Leishmania infection may take a course along the lymphatic vessels and produce small nodules round the original lesion (metastasis in the skin), as well as infections of the regional lymph-nodes.

2. When the infection travels via the lymphatic channels, nodes are liable to occur, i.e., bubonuli leishmaniae.

3. In several cases, a considerable time after the original leishmania lesion had healed, either an inflammatory reaction of a tuberculous form developed in the vicinity, or the abortive elements found at the periphery began to grow.

4. The eruption occurs in various forms : (*a*) Nodes may be found isolated beyond the edge of the leishmania scar (leishmania recidiva nodosa) ; (*b*) Nodules in a circular form may be found round the scar (leishmania recidiva orbicularis) ; (*c*) Tubercles may be grouped in multiple semicircles, which heal from within and leave scars, while the periphery progresses slowly (leishmania recidiva tubero-serpiginosa).

5. The proof that this is a special form of leishmania is based on the following :

(*a*) The clinical appearance of a morphological unit.

(*b*) Positive intradermal reaction of L.V.R.

(*c*) Findings of parasites in the lesion in one case.

(*d*) The histological study revealing the characteristic structure of leishmania lesions.

(*e*) Lack of positive findings for lupus or tertiary lues.

(*f*) The pathogenicity of all these cases consists of the disturbance of the immunity, in consequence of improper treatment, and lack of resistance in asthenic, poorly nourished individuals.

CONCLUSION

All these cases described above exhibit forms of leishmania which may be termed leishmania recidiva.

I am indebted to Professor S. Getzova, of the Department of Pathology of the Rothschild-Hadassah Hospital, Jerusalem, for her assistance.

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EXPLANATION OF PLATE IX

Fig. 1. Bubonuli leishmaniae (the arrows point to three nodules below the ulcerating leishmania lesion on the cheek and to a swollen lymph-channel).

Fig. 2. Case 1 : Leishmania recidiva orbicularis (on the forearm are nodules arranged in a circle round an old leishmania scar).

Fig. 3. Case 2 : Leishmania recidiva tubero-serpiginosa (on the nasal side of the leishmania lesion healing is taking place, while on the side of the cheek a progressive semicircular eruption of tubercular form is to be seen).

Fig. 4. Case 3 : Leishmania recidiva lichenoides polycyclica (round the old leishmania scar is a polycyclic strip consisting of a small papulo-tubercular eruption).

Fig. 5. Leishmania recidiva nodosa (note a new leishmania tubercle near the old leishmania scar, and a second one in the sound skin at a distance from the first).



FIG. 1

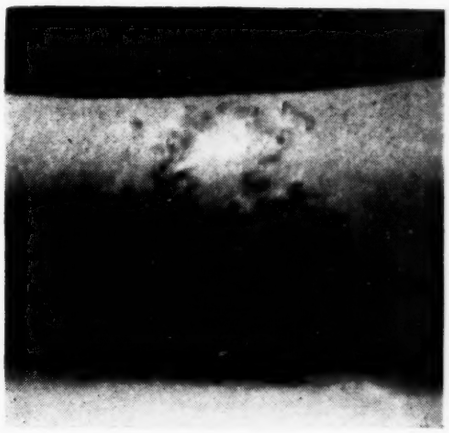


FIG. 2



FIG. 3

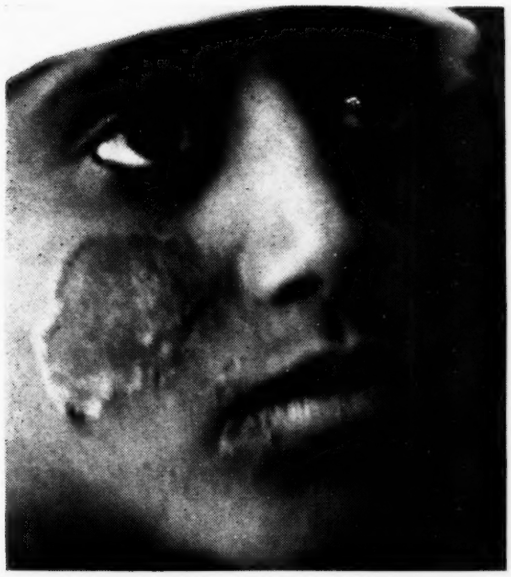


FIG. 4



FIG. 5

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ON A MELANIC INLAND RACE OF *ANOPHELES COSTALIS* GILES (*GAMBIAE*) IN UGANDA

BY

E. G. GIBBINS

(*Received for publication* 12 *June*, 1936)

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INTRODUCTION

The purpose of the present paper is to give an account of some observations made in Uganda concerning the melanic propensities of a race of *Anopheles costalis*.

As long ago as 1903 Theobald drew attention to a ' marked melanic variety ' of *Anopheles costalis* from Bathurst in Gambia, which he described as *costalis* var. *melas*. Since that time, several instances of the occurrence of dark forms of *costalis* have been recorded from different parts of Africa, but in each case the insect has originated from the region of the coast. No investigation of the phenomenon was undertaken until 1930, when Barber (1931) made some interesting observations in Southern Nigeria. His material, together with two adults from Dar-es-Salaam in Tanganyika Territory, was studied in detail by Dr. Evans, of Liverpool, who subsequently (1931) published a full account of the investigation. The outcome of these studies was the association of melanism in *costalis* with adults which had been reared from larvae taken in brackish water with a high degree of salinity. It is not without interest, therefore, to record the occurrence of an inland race showing melanic tendencies, whose early stages were not associated with saline waters.

OBSERVATIONS IN UGANDA

APRIL 5th, 1936. Two rain-water rock-pools situated near the Sezibwa Falls on the edge of forest, but far enough away to expose them to full daily sunshine, some 30 miles east of Kampala, furnished a supply of melanic larvae. The pools were small and shallow (2 feet long by 6 inches broad by 3 inches deep) with a rich black loam on the bottom ; they were situated about a foot apart, and were actually small fissures in a sloping rock. Each had a tuft of grass at one end, and contained frog-spawn and tadpoles. Though the pools were near together, and though in each case the water was on the acid side (pH 6·8) and showed a slight trace of dissolved iron, the amount of chlorides was entirely different, 6 parts per million being present in the one pool and 16 parts in the other.

The larvae were black and showed extensive markings of the fronto-clypeus

(fig. *b*) similar to the melanic specimens from Butiaba but not so deeply pigmented ; and the bred adults, 6 males and 11 females, which showed no difference on account of their being in different pools, did not exhibit the same marked degree of melanism. The larval skins had a brown pecten, heavily chitinized tergal plates and a fronto-clypeus as mentioned above. One male showed a distinct reduction in the size of the pale rings of the hind tarsi, and its larval skin showed a lightly pigmented fronto-clypeus. In the case of one female the fronto-clypeus was identical with the Butiaba specimens (fig. *c*).

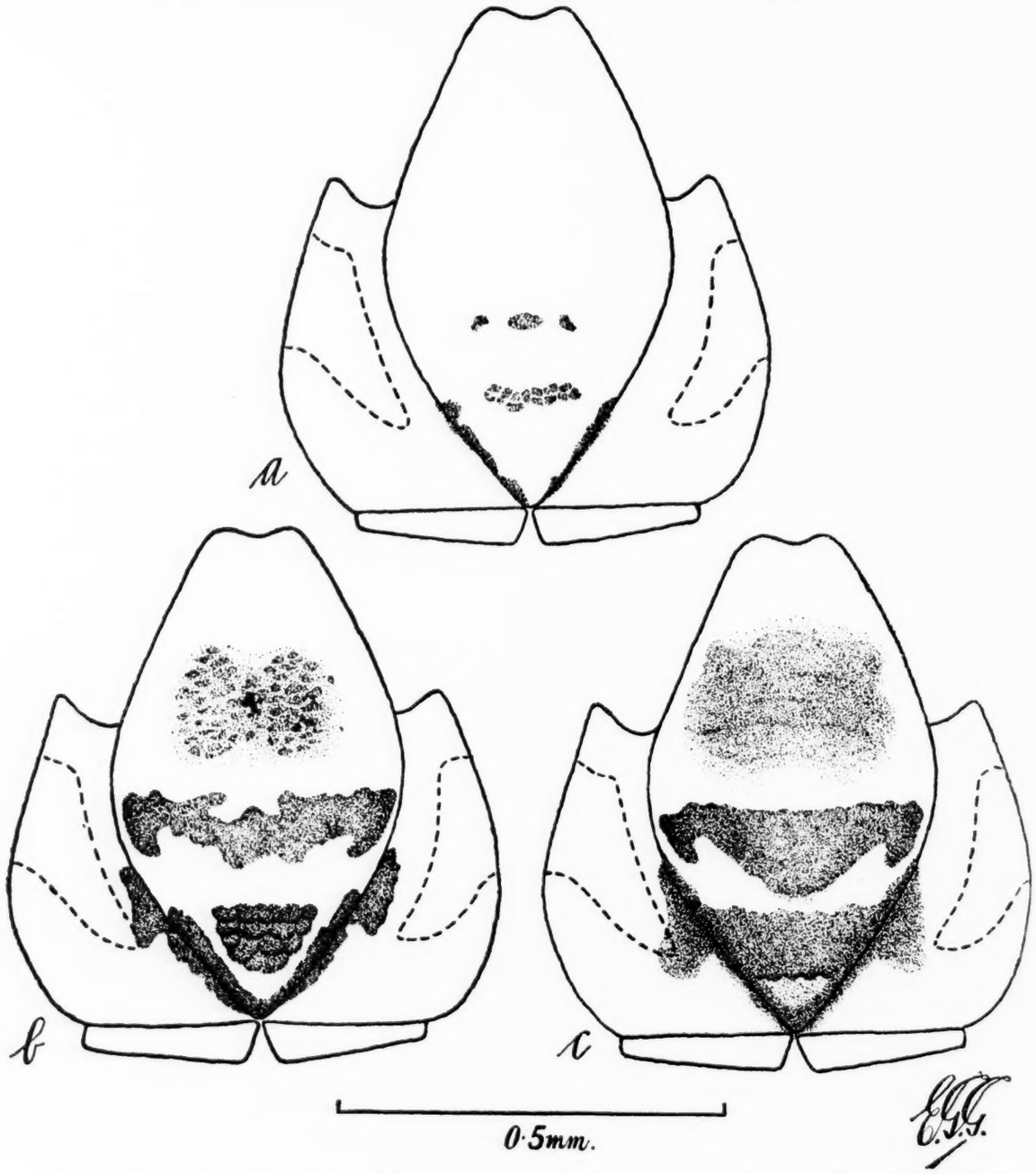


FIG. Head of larva of *Anopheles costalis* drawn to show the pigmentation of the fronto-clypeus. *a*.—Normal *costalis*, drawn from Kampala specimen ; *b*.—Melanic race, Sezibwa form ; *c*.—Melanic race, Butiaba form.

April 11th, 1936. Numerous jet-black larvae of *Anopheles costalis* were collected from a rain-water pool in an open swampy area behind the foreshore of Lake Albert, near Butiaba. The breeding water was slightly alkaline (pH 7·6) and had the appearance of strong tea. It contained a slight trace of iron, and chlorides expressed as Cl were present to the extent of 4·9 parts per million. Though the neighbourhood was covered with innumerable pools of varying size, all equally exposed to the sun, *costalis* larvae were only found in one, about 8 inches deep, which was partly covered by a blue-green scum. The pool had an underlying layer of thick black mud, and was situated beside a well-trodden path leading from a native village to open water. Apart from their very black appearance, the larva differed from normal *costalis* in the fronto-clypeal markings (fig. *c*), and when examined microscopically the pigmented pattern of the head was most striking. This character was a constant feature of the 36 larvae collected.

Seven mature specimens were isolated the same evening and gave rise, 5 days later, to 3 males and 4 females with the integument of the thorax and abdomen very dark. On April 14th, 2 more larvae were isolated, and 4 days later a single male and female emerged ; these two adults, unlike those from the original isolations, were not so dark and differed little from normal *costalis*. The larval skins showed heavily chitinized tergal plates, a brown pecten and a deeply pigmented fronto-clypeus ; in 8 cases the dorsal surface of the head resembled fig. *c*, while in one skin, that from which the last female emerged, it appeared as depicted in fig. *b*. An inspection of 5 remaining larvae on April 19th, 8 days after collection, showed the loss of all dark pigmentation with the exception of the head, where the markings showed no alteration from their original appearance. While in captivity the larvae had been kept in their original breeding water and had been given a daily supply of fine yeast.

April 13th, 1936. For control purposes, larvae were taken from a typical *costalis* breeding place—a clay pit—at Bukomero, a small native village situated 55 miles from Kampala. The larvae were developing in muddy water, 6 inches deep, which had an underlying layer of thick brown mud. The water contained a slight trace of iron, as in the case of that from Butiaba, but was acid in reaction (pH 6), and chlorides expressed as Cl were present to the extent of 1 part per million. The larvae were a greenish-brown colour, devoid of dark pigmentation and fronto-clypeal markings.

Eight isolated larvae gave rise to 4 males and 4 females. The integument of the thorax and abdomen of the adults was brown and the larval skins were pale ; the tergal plates were hardly visible, and the pecten was a pale yellow, while there was no sign of any markings on the fronto-clypeus.

April 24th, 1936. Further control specimens from a typical *costalis* breeding place were obtained. A pool in a ditch situated below a potato-patch on the outskirts of Kampala, which was replenished from time to time with water overflowing from a native well, furnished 119 light-brown *costalis* larvae. The

bottom of the pool was covered with a thick brown mud, and over its surface ran scattered streamers of a creeping grass. The muddy water, which was slightly alkaline (pH 7·2), contained the usual slight trace of iron and an exactly similar amount of chlorides as was found in the Butiaba water (16 parts per million) which supplied the melanic specimens.

From isolated larvae, 9 males and 15 females were bred. In five cases the fronto-clypeus of the larval skins were devoid of pigmentation, in 14 specimens the markings were much reduced and resembled fig. *a*, while in three instances the pigmented areas closely approached in size those from the Sezibwa rock-pools, as depicted in fig. *b*. The adults of this series showed the light-brown coloration of the thorax of normal *costalis*, but a number of specimens (in addition to the 2 females which were bred from the larvae showing the melanic type of fronto-clypeus) exhibited a dark-grey thorax.

DISCUSSION

The study is based on the critical examination of the larval skins, pupal pelts and adults of 56 isolated individuals obtained from four different and widely separated localities, details of which have been given above and are summarized in Tables I and II. In addition, a large number of mature larvae have also been studied. The present investigation has confirmed the opinion of Edwards (1912) that *Anopheles costalis* is a variable species. Dr. Evans's illustration of an adult in her ' Guide to the Anophelines of Tropical and South Africa ' is an accurate picture of the common form of the female found in Uganda, which is normally rather darker than those found elsewhere in Africa. The pale interruption in the third main dark area of the first vein may be entirely absent, and occasionally the upper branch of the fifth vein is wholly dark. Sometimes the markings of both wings do not coincide. Theobald (1901) noted that examples from Central Africa had a long wing. The palps do not show much variation, though in one series the middle pale band was somewhat inconspicuous. A single specimen agreeing with ' *costalis* var. *quadriannulatus** ' Theobald, which was taken during a mosquito survey of Jinja in 1930, is the only record of this variety from the country. In the case of the specimens bred in connection with this study, the speckling of the legs was generally well marked, but it has been noted that examples captured in human habitations in the course of mosquito surveys may show little evidence of this character, particularly if they have become badly rubbed.

Apart from a general darkening of the integument of the thorax and abdomen, the melanic inland race occurring in Uganda shows no morphological differences either externally or in the male terminalia from normal *costalis*. There is no tendency to a reduction in the tarsal rings, and in this character the Uganda specimens resemble those described by Dr. Evans from Dar-es-Salaam.

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*Dr. Evans very kindly confirmed the writer's determination of this specimen.

The larva of *Anopheles costalis* has been dealt with by Macgregor (1927), Evans (1927), De Meillon (1931) and Symes (1932); but here again, as stated by Wilson (1936), it is subject to a good deal of variation. A small degree of variation in the small setae of the head is common to most *Anopheles* larvae, but, in addition, in normal *costalis* the palmate bristles vary in shape, the relative size of the shoulder hairs differ, while the pecten varies in the number and in the arrangement and comparative length of the long and short teeth. Nevertheless, there is one character in which the larvae of the melanic race of *Anopheles costalis* may be distinguished from the normal individual, i.e., the striking pigmentation of the fronto-clypeus, which appears to conform to a regular pattern (fig. *b*, *c*).

In agreement with the findings of Dr. Evans (1931), the pecten was more heavily chitinized in the melanic specimens, but only in the case of the Butiaba specimens was there a definite irregularity in the length of the teeth. In typical *costalis* in Uganda the degree of pigmentation of the tergal plates varies, and in some skins from normal examples the plates were equally as dark as in the melanic race. The pupal pelts from normal and melanic specimens were identical, and those of the latter showed no undue darkening.

TABLE I

Giving details of the breeding places and breeding waters

Date	Locality	Breeding place	Breeding water	pH	Chlorides expressed as Cl, parts per million	Condition of larvae
Apr. 5th, 1936	Sezibwa Falls, 30m. E. of Kampala	Two small pools in sloping rock	Clear and colourless	6·8	6	Melanic
				6·8	16	Melanic
Apr. 11th, 1936	Butiaba (2,028 ft.) L. Albert	Small pool in swamp	Peaty and clear (colour of strong tea)	7·6	4·9	Melanic
Apr. 13th, 1936	Bukomero, 55m. N.W. of Kampala	Small pool in deep clay pit	Muddy	6	1	Normal
Apr. 24th, 1936	Kampala (3,800 ft.)	Small pool in ditch	Muddy	7·2	16	Normal

Dissolved iron was present in all the waters in minute quantities.
The pH readings were made on the waters after collection and not on the spot.

The chemical constituents of the breeding waters are summarized in Table I. The amount of chlorides present was never more than 16 parts per million. In two instances it was equal in amount in two entirely different types

of breeding waters: one a rock-pool at the Sezibwa Falls, which furnished melanic larvae, and the other a ditch-pool at Kampala, where mainly normal larvae were obtained. The adults showing the most marked degree of melanism came from Butiaba, where the water contained only 4·9 parts of chlorides per million. Dissolved iron was present in very minute quantities in all the waters. It is therefore clear that the melanic tendencies of *Anopheles costalis* in Uganda are not due to the salinity of the breeding waters or to the presence of dissolved iron.

It appears to be a question of the type of food which in certain cases may be influenced by chemical factors. All the pools in the Butiaba swamp were peaty to an extreme degree, and only in the case of the one which had a food-supply in the form of a blue-green scum were melanic larvae found. In this connection it may be mentioned that the Dar-es-Salaam specimens* of melanic *costalis* determined by Dr. Evans also came from a peaty rain-water pool in a swamp. That food is an important factor is shown by the fact that in the presence of a daily supply of finely powdered yeast the Butiaba larvae, which had been kept in their original water, lost most of their melanic pigment in the course of 8 days. It was in the case of those larvae which pupated soon after collection, and did not reap the benefit of an artificial food-supply, that the adults were most markedly melanic. The finding of melanic larvae in the Sezibwa rock-pools

TABLE II

Summary of details concerning the isolated adults and their respective larval skins

Locality	Pigmentation of the fronto-clypeus of larval skins as in				Adults		Remarks
	Fig. *a*	Fig. *b*	Fig. *c*	Without pigmentation	♂♂	♀♀	
Sezibwa	1	15	1	—	6	11	Adults similar to Butiaba specimens but not so markedly melanic
Butiaba	—	1	8	—	4	5	Thorax and abdomen markedly melanic
Bukomero	—	—	—	8	4	4	All normal *costalis*
Kampala	14	3	—	5	9	15	Of the 3 adults (1♂ and 2♀♀) with the fronto-clypeus as in fig. *b*, the two females showed a dark-grey thorax and the male a marked reduction in the sub-terminal dark band of the palps. Several other adults of this series were also somewhat dark

*Recently *Anopheles costalis*, determined as var. *melas*, has been found breeding in highly saline water in Dar-es-Salaam (Tanganyika Territory medical report for 1934).

is puzzling, but it is quite possible that they were not in their true environment at the time of collection, as there had been recent heavy rain. There was no lack of food in the muddy pools of Bukomero and Kampala, in which normal *costalis* thrived. They had a large microfauna, and the larval skins from which adults had emerged showed numerous plankton clinging to the pelts.

While the fronto-clypeus of the head of normal larvae from Bukomero was devoid of markings, there was a small amount of pigment present in the majority of control specimens from Kampala. Apart from a few instances it was confined to the proximal half of the fronto-clypeus and was present in small areas (fig. *a*), but in three examples the pigment closely approached the dimensions and degree of chitinization present in melanic specimens. In the presence of a plentiful food-supply the adults, one male and two females, differed little from normal *costalis*. However, the females exhibited a dark-grey thorax, but some other members of this series without the deeply pigmented fronto-clypeus of the larva also showed the same degree of darkening.

SUMMARY

1. The paper records the finding of a melanic inland race of *Anopheles costalis* in Uganda.

2. Melanism was most marked in those adults which were bred from larvae taken in peaty water.

3. When kept in their original breeding water and given an artificial supply of food in the form of finely powdered yeast, the melanic larvae lost most of their dark pigmentation.

4. The larva of the melanic race can be easily distinguished from normal *costalis* on the character of the fronto-clypeus, which does not appear to alter even in the event of the larva subsequently losing its dark pigment.

5. Individual larvae showing the characteristic fronto-clypeus of the melanic race may occur amongst normal examples from typical *costalis* breeding places. When reared from this type of habitat where food is abundant, the adults do not exhibit melanic pigmentation.

ACKNOWLEDGMENTS. It is a pleasure to record my indebtedness to Dr. Alwen M. Evans, of the Liverpool School of Tropical Medicine, who, during a hurried visit to Uganda, not only found time to read through the manuscript of this paper but also looked over the writer's material and made helpful criticisms. My thanks are also due to Mr. H. S. Leeson, who perused the manuscript while in Uganda conducting mosquito researches on behalf of the London School of Hygiene and Tropical Medicine ; and to Mr. J. S. McDonald of the Uganda Medical Laboratory Services, who spontaneously undertook the chemical analyses of the breeding waters.

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THE NUCLEAR DIVISION OF *DIENTAMOEBA FRAGILIS*

BY

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Most of the authors who have studied *Dientamoeba fragilis* morphologically have used faecal smears as material. To judge from the experience gained with other intestinal amoebae, phases of nuclear division could hardly be expected in such material. Jepps and Dobell (1918) figured one amoeba with an abnormal nucleus which they suggested might be a dividing one.

In slides prepared from a pure culture of *Dientamoeba fragilis* and stained with Heidenhain's haematoxylin I found a great number of amoebae with abnormal nuclei. As far as I can judge they cannot be interpreted otherwise than as stages of nuclear division. Amoebae in the act of plasmotomy were never found, either in the slides referred to or in others ; at the moment I am unable to explain this.

Fig. 1 represents a uninucleate amoeba with a nuclear structure as commonly found in faecal smears (a ' common ' nucleus) ; the chromatine granules are embedded in a matrix of globular shape, staining grey, the whole surrounded by a clear halo bordering on the protoplasm. I must confess that it was only on very rare occasions that a distinct nuclear membrane could be distinguished between the concentric clear space and the protoplasm. The first change shown by the dividing nucleus is its becoming oval in shape (fig. 2) ; then it elongates more and more (figs. 3, 4, 5 and 6). Simultaneously the clear space between matrix and protoplasm disappears, and the matrix comes into close contact with the protoplasm. Towards the end of this phase of nuclear elongation the number of chromatine granules has increased considerably. Whereas in the ' resting ' nuclei it is 3–7, it has risen to 6–14 in the nucleus which has attained its maximum length. Next the granules arrange themselves into two separate groups (fig. 7), the space between these groups becomes paler (fig. 8) than the parts of the matrix enveloping the granules, and the matrix completely breaks up into two parts (figs. 9, 10). Now the clear space around the matrix reappears (fig. 11, in one nucleus only ; figs. 12 and 13, in both nuclei), the nuclei still remaining oval in shape. Finally they assume the ' normal ' globular shape (fig. 14). It is true that in this system of nuclear division such figs. as 12 and 13 might well be interpreted as representing amoebae in which the two nuclei are preparing for a second division (compare fig. 13 and fig. 2), resulting in a quadrinucleate amoeba. As a matter of fact, quadrinucleate amoebae do occur (fig. 19), but they are so exceedingly rare that it seems more rational to consider fig. 12 and fig. 13 as telephases than as prophases.

Wenrich (1936) has drawn attention to the fact, already recorded by Jepps and Dobell (1918) and by Wenyon (1926), that in many amoebae one of the chromatine granules exceeds the others in size and stains more deeply. In my slides I found this amply confirmed (figs. 15, 16, 17). In many amoebae the nucleus even showed one well-stained granule separated from the crescent-shaped matrix by a concentric pale band (fig. 18), thus resembling the nucleus of *Iodamoeba bütschli*. This nuclear picture occurred so often in my slides and was so characteristic that the possibility must be considered of two species of amoebae having developed in the cultures. However, every kind of transitional form between such nuclei as are figured in fig. 18 and those represented in fig. 14 could easily be found, and it seemed more rational to consider all these forms as belonging to one species.

Wenrich (1936) considers the main granule as a karyosome. In some of the dividing nuclei it is rather conspicuous (figs. 3, 4, 6), but I found no indication that it plays a particular rôle during nuclear division, which seems contrary to Wenrich's thesis.

At first sight the nuclear division described above might be considered as an instance of amitosis. It is quite possible, however, that owing to the smallness of the objects certain features have been overlooked which might alter this supposition.

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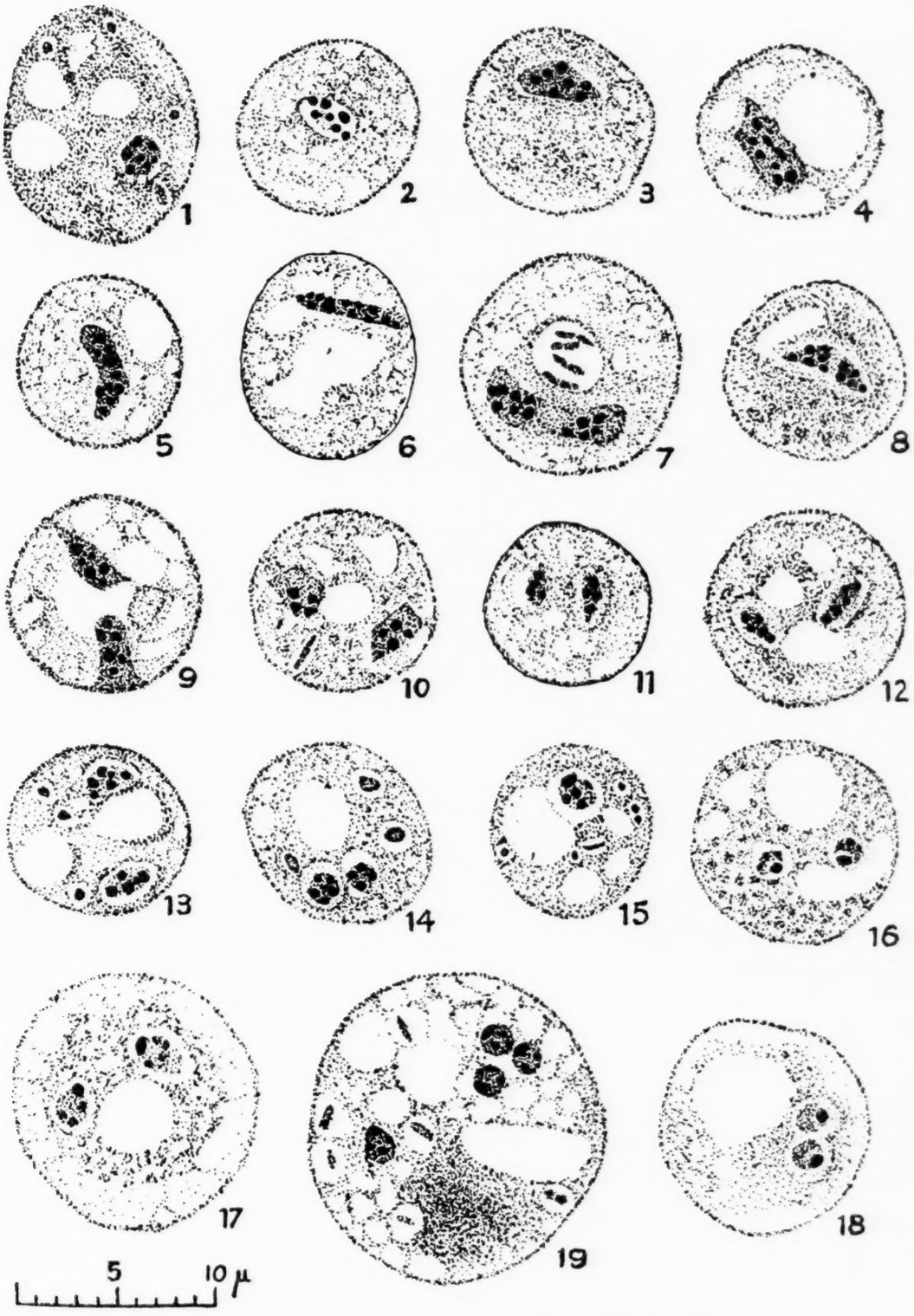


Fig. showing the nuclear division of *Dientamoeba fragilis*.

THE ANTIGENIC COMPOSITION OF *TRYPANOSOMA EVANSI*

BY

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AND

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Pathogenic protozoa differ essentially from bacteria both in their pathogenicity and in the immunity response which they elicit. Recently reported experiments (Kligler and Comaroff, 1935) have shown that the injection of a suspension of dead trypanosomes produced a partial immunity in animals. Subsequent experiments indicate that this immunity is strain specific.

The reason for this peculiar lability of protozoa and the limited specificity has not as yet been elucidated. For obvious reasons it has been difficult to apply the newer methods of antigenic analysis to protozoa. Such an analysis requires large amounts of material, which can only be obtained by mass cultures of the organism on artificial media. This method is not applicable to protozoa. We have been able to overcome this difficulty by using the technique developed for the preparation of trypanosome vaccine for immunity studies (Kligler and Comaroff, 1935). Using the rat as a culture medium, we have been able to obtain sufficient material for a quantitative estimation of the composition of *Trypanosoma evansi*. The present paper presents the results of a preliminary series of experiments with this organism.

Technique. Rats infected with *T. evansi* were bled shortly before death, when the number of organisms was approximately 1,000,000 per c.mm. of blood. The rats were bled from the heart into a 1 per cent. glucose citrate solution, about 1 c.cm. of blood being added to 9 c.cm. of the citrate solution. The mixture was first centrifuged at low speed (500 revolutions per minute) to remove the red cells. The turbid supernatant fluid was then removed to another tube and centrifuged at high speed to throw down the trypanosomes. The sedimented trypanosomes were washed with saline and centrifuged, the clear supernatant fluid pipetted off and the packed sediment dried *in vacuo* over $CaCl_2$.

The material obtained from 10 large rats weighed about 50 mg., an amount sufficient for the various analyses and tests. Since the trypanosome suspension always contains a certain number of white cells, control material was prepared from normal rats in order to ascertain the extent of the error. The weight of white cells obtained in this way proved so small, in relation to that of trypanosomes, as to be negligible.

Antigenic analysis. The analysis was made in the following manner :— The dried material was suspended in alcohol, in the proportion of 25 c.cm. of 96 per cent. alcohol to 50 mg. of substance, and extracted for two days at 30° C. The suspended material was then filtered through filter-paper and the

residue re-suspended in 15 c.cm. ether and extracted for 48 hours at 20° C. The ether was removed by filtration and the residue dried and weighed. It was then taken up in N/2NaOH, heated to 37° C. and filtered. The solution was then treated twice with two volumes of absolute alcohol, and the second precipitate, which represented the C or carbohydrate substance, was centrifuged, dried and weighed. In another lot the residue obtained after the alcohol and ether extraction was analysed for N.

This procedure was unsatisfactory because relatively large amounts of the dissolved material were lost in the filter. The analysis was, therefore, repeated, using centrifugation instead of filtration.

In this manner four fractions were obtained : (1) Alcohol soluble ; (2) Ether soluble ; (3) The protein residue ; (4) The C or carbohydrate fraction.

For purposes of comparison an analysis was made by the same procedure of a strain of Shiga dysentery bacillus.

The results of the analyses are shown in the Table.

TABLE

Comparison of composition of *T. evansi* and *B. shiga*

Analysis no. 1	Weight of material treated ; mg.	Weight of residue after alcohol extraction ; mg.	Weight of residue after ether extraction ; mg.	Weight of C substance from residue ; mg.	Protein in residue after alcohol-ether extraction (percentage)
Trypanosome					
Weight	50·0	26·0	21·0	1·0	
Percentage of mass ...	100·0	52·0	42·0	2·0	25·0
B. shiga					
Weight	75·0	66·0		12·0	
Percentage of mass ...	100·0	88·0		16·0	44·0

Analysis no. 2	Weight of material treated ; mg.	Weight of alcohol extract ; mg.	Weight of ether extract ; mg.	Weight of of residue ; mg.	Weight of unextracted material ; mg.	Weight of C substance from unextracted cell ; mg.
Trypanosomes						
Weight	23·0	10·0	3·0	9·0	38·0	2·5*
Percentage of total	100·0	43·5	13·0	39·1	100·0	6·6
B. shiga						
Weight	40·0	4·0	2·0	33·0	57·0	8·0
Percentage of total	100·0	10·0	5·0	82·5	100·0	14·0

*It should be noted that this substance differs from that obtained from bacteria in that it is not readily hydrolyzable with HCl. Its nature and antigenic properties are being investigated. Heating 2 hours with 2N/HCl gave partial hydrolysis and only slight reduction with Fehling solution.

There was close correspondence in the results of the analysis by the two procedures. In the first instance the residue of the trypanosomes after alcohol and ether extraction amounted to 42 per cent. of the total, and by the second procedure to 39 per cent. In the case of the Shiga bacillus, the alcohol extract amounted to 12 per cent. in the first and to 10 per cent. in the second analysis.

There was a larger difference in the C substance in so far as the trypanosomes were concerned. In the first series the C substance was prepared from the residue after extraction, and in the second from the whole trypanosome. It is possible, therefore, that in the second instance substances other than carbohydrates were dissolved by the NaOH and precipitated by the alcohol. In the case of the Shiga bacillus, the amount of C substance was essentially the same whether prepared from the extracted residue or from the whole bacteria. The C substance from trypanosomes is only partially hydrolyzed with HCl, and that only after prolonged treatment.

The differences in the composition of the two types of organisms are apparent. In the bacterial cell alcohol and ether extractable lipoids account for about 12–15 per cent., C or carbohydrate fraction for 14–16 per cent., and protein for 44 per cent. of the cell mass ; in the trypanosome cell the alcohol and ether extractable lipoids constitute 56–58 per cent. of the cell mass ; the C or carbohydrate fraction only about 2 per cent., and the protein 25 per cent. Three-fifths of the bacterial cell are protein and carbohydrate, while three-fifths of the trypanosome cell are lipoids.

Toxicity of fractions. There have been basic differences of opinion concerning the toxins produced by trypanosomes and the toxic character of these cells. Previous attempts to determine the toxic effect of dead trypanosomes have yielded negative results (Kligler, Geiger and Comaroff, 1929). We restudied this question by testing the toxicity of the various fractions in relation to the Schwartzman phenomenon. Each of the fractions was used both as local, skin preparatory and as general inciting agent. *Eberthela typhi* was used as control.

The following experiment is illustrative. The abdomens of four rabbits were shaved, and the following substance was injected intradermally, each at a distinct point : (1) The alcoholic extract dried and resuspended in saline ; (2) The alkali-soluble (or C) fraction ; (3) The protein residue ; (4) The typhoid toxic fraction (P_2). On the next day the rabbits were given one of the substances intravenously ; each rabbit received a different substance. The results were entirely negative. The only point positive was that prepared with the typhoid toxic fraction in the rabbit receiving the P_2 fraction intravenously.

It is apparent, therefore, that the various fractions of the trypanosome cell are non-toxic. They possess neither skin preparatory potency nor toxic potency when given intravenously.

DISCUSSION

Although these studies are still in an early stage, the results obtained thus far throw interesting light on the unique antigenic character of trypanosomes. It is known that pathogenic protozoa possess two distinguishing characteristics : (1) they are poor antigens and produce at best a low-grade and relatively transient immunity ; (2) they are extremely labile and readily develop serum and drug-fast variants.

Both of these characteristics may properly be attributed to the predominance of lipoids in the cell. The recent studies on bacterial antigens have demonstrated that the specificity of the organisms is determined by the carbohydrate (glucoside) constituents of the cell, and that these substances (haptens) associated with a protein constitute highly active antigens. The one group of pathogenic bacteria of low antigenic quality are the tubercle bacilli, known to have a high lipoid content.

Levene, Landsteiner and van der Scheer (1927) have demonstrated that lipoids, even when impure, are poor and uncertain antigens. These authors have obtained variable results with lecithins from various sources. They have also shown that, although when combined with serum proteins they may serve as antigens, the antibody response was low and highly variable.

Only few studies have been made on the immunity response to dead protozoan antigens, and these relate to their ability to produce sera giving a positive Wassermann reaction. Klopstock (1926) immunized animals with *T. pallida* and obtained a positive Wassermann reaction only when very heavy suspensions of spirochaetes were injected. He further showed that the alcohol extracts of the spirochaetes served equally well as a Wassermann antigen as organ lipoids. Landsteiner and van der Scheer (1926) inoculated rabbits with large doses of *T. equiperdum*, and the animals developed both positive Wassermann and Sachs-Georgi reactions. The animals treated in this way did not fix complement with lecithin, whereas those infected did ; the former fixed complement with alcoholic extracts of trypanosomes. Preliminary experiments carried out by us with the various fractions showed that the injection of the lipoid fraction induced a positive Wassermann reaction in rats, but did not confer any immunity to an infection.

It appears that the peculiar antigenic character of these organisms is bound up with the lipoid constituents of the cell. The quantitative data given above indicate that the lipoid is the principle fraction of the cell and, consequently, probably the dominant antigen. At the moment little is known of lipoids, their antigenic character and specific properties. It seems, however, reasonable to postulate that the lability of these organisms, their rapid strain modifications in different hosts, and their ability to form serum- and drug-fast strains are in some way associated with the lipoid antigens and their ability to undergo qualitative, quantitative or stereometric changes. In other words, the antigenic fixity of the bacterial cell is attributable to the dominance of carbohydrate

haptens, while the antigenic lability of certain protozoa (*T. pallidum*, pathogenic trypanosomes, plasmodia, etc.) is probably due to the predominance of the lipoid haptens.

It is also of interest to note that in contrast to most bacteria the trypanosome is devoid of endotoxin. Its pathogenicity is, apparently, referable to its low antigenicity and high physiological activity—more particularly to the high glycolytic activity of the organisms (Yorke, Adams and Murgatroyd, 1929; Kligler, Geiger and Comaroff, 1929).

CONCLUSIONS

The trypanosome studied (*T. evansi*) differs basically in its antigenic composition from bacteria: nearly 60 per cent. of the trypanosome cell is lipoidal in nature and extractable with alcohol and ether and only 2–6 per cent. C substance; in contrast to this, the bacterial cell contains 14–16 per cent. C substance and only 12–15 per cent. lipoids.

The C substance of bacteria is readily hydrolyzed by N/1HCl in 30 minutes, while that of the trypanosome is only partially hydrolyzed by heating for 2 hours with 2N/HCl.

The various fractions of the trypanosome are non-toxic as tested by the Schwartzman phenomenon.

The injection of the lipoid fraction into rats produces a positive Wassermann reaction, but does not enhance resistance to infection.

It is suggested that the antigenic peculiarities of the trypanosome are ascribable to the lipoids which constitute the major part of the cell.

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ON THE NOMENCLATURE AND SYNONYMY OF THE NEMATODE '*SETARIA LABIATO-PAPILLOSA*'

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For more than half a century the name *Filaria* (or *Setaria*) *labiato-papillosa* has been in general use for a well-known parasite of cattle and other ruminants. Stiles and Hassall (1920), in a footnote to the entry *Filaria labiato-papillosa* in their valuable 'Index-Catalogue' (p. 458), say: 'This specific name is attributed by authors to Aless., 1838, Nuovi annali delle Sc.nat., but we do not find it here.' Sherborn (1927, p. 3331) was likewise unable to find this name mentioned by Alessandrini in the place cited. The writer has also searched for it there in vain, and indeed has been unable to trace any reference to the use of the name by Alessandrini.

Perroncito (1882, p. 326) describes the species under the heading of '*Filaria labiato papillare*,' prefacing his account of it with the following words: '*Filaria labiato papillosa* ; *filaria labiato papillare*, Alessandrini.—È tutto merito del grande Alessandrini l'aver distinto questa specie di filaria dalla precedente (*F. papillosa*)* . . .'

It will be observed that Perroncito here gives no date or reference for Alessandrini. However, in the second edition of his textbook (1901, p. 403) he gives '*Filaria labiato-papillare* (*Filaria labiato-papillare* Alessandrini, 1838 ; *F. cervina* Duj., 1845 ; *F. terebra* Dies., 1851),' and repeats the statement as to Alessandrini having distinguished the species from that of the horse. This is the earliest reference that the writer has been able to find in which a name resembling, though not identical with, '*labiato-papillosa*' is definitely attributed to Alessandrini. Stossich (1897, p. 41) quotes Alessandrini as the author of the name *labiato-papillosa*, and gives a reference to the 1838 publication. Railliet (1885, p. 378) refers to 'Filaire bovine (*F. terebra* Dies., *F. cervina* Duj.)', but three years later (1888, p. 98) he writes 'des Filaires labiopapilleuses (*F. labiato-papillosa*† (Alessandrini, *F. terebra* Dies., *F. cervina* Duj.),' while in the second edition of his textbook (1893, p. 526) he writes 'Filaire à lèvres papilleuses (*F. labiato-papillosa* Alessandrini, 1838.—Syn. : *F. cervina* Duj., 1845 ; *F. terebra* Dies., 1851).' The authors of numerous other textbooks and papers have

*Perroncito's description of the *Setaria* of the horse (*loc. cit.*, p. 325) is headed '*Filaria papillare*,' with the sub-heading '*Filaria papillare* (*Filaria papillosa*, Rud.).'

†This seems to be the first appearance of the hyphenated name.

similarly ascribed the name to ' Alessandrini, 1838,' without giving any further clue to the original reference.

Evidence has not been forthcoming to show that the name ' *labiato-papillosa* ' (with or without the hyphen) was published earlier than by Perroncito (1882). Even if it should ultimately prove that Alessandrini was responsible for it, an earlier name seems to be available for the species, and should, in the writer's view, be applied to it in accordance with the law of priority. Rudolphi (1819, p. 8) uses the name *Filaria cervi* for a worm in the Vienna Museum from the red deer (*Cervus elaphus*), and the name *Filaria bubali* for a similar worm from a buffalo (*Bos bubalus*). It is true that he gives no morphological description of the species, and both names are marked with a †, indicating that Rudolphi himself had not seen the specimens. Nevertheless, the citation of the hosts, and of the position of the worms in the hosts, might, in 1819, have been considered a sufficient ' definition ' to satisfy the conditions of Article 25 of the International Rules of Zoological Nomenclature. It does not, therefore, seem necessary to dismiss the names at once as *nomina nuda*.

The identity of the species referred to by Rudolphi as *Filaria cervi* seems to be established, with very little room for doubt, by Dujardin (1845, p. 49), to whom we owe the first description of the parasite. This author, after his description of *F. cervina*, adds the following note : ' Le muséum de Vienne a envoyé, en 1816, à celui de Paris, l'helminthe que je décris ici et qui avait été trouvé dans l'abdomen d'un cerf (*Cervus elaphus*). . . .' From this statement, in view of the date and the host, it appears almost certain that the material described by Dujardin was the same, or part of the same, as that recorded, but not described, by Rudolphi in 1819. This strengthens the writer's view that Rudolphi's name *cervi* may legitimately be used for the species. If this reasoning be not accepted, and unless it can be shown that the name *labiato-papillosa* was actually published before 1845, then the next available name in order of priority appears to be *cervina* Dujardin, while the claim of *terebra* Diesing, 1851, would have to be considered before the ' *labiato papillare* ' or ' *labiato papillosa* ' of Perroncito could be adopted.

In connection with the synonymy of the species, several forms described since 1851 must be considered.

1. *Setaria bidentata* (Molin, 1858), Railliet and Henry, 1911. Molin (1858, pp. 401, 405) divided Diesing's *Filaria terebra* into two species. The material recorded by Diesing from *Cervus elaphus* was regarded as the true *F. terebra* (p. 405), while the specimens from Brazilian deer (*Cervus simplicicornis*, *C. rufus* and *C. nambi*) were re-examined by Molin and described by him as a new species under the name of *Filaria bidentata* (p. 401).

Molin's description of the latter contains nothing that conflicts with our present conception of ' *S. labiato-papillosa*,' and indeed he himself states that the species is distinguishable from *Filaria terebra* only by the presence of two chisel-shaped (*meisselförmiger*) teeth. Cameron (1936) has examined specimens from *Mazama simplicicornis* from Trinidad, which he assigns to *S. bidentata*, and gives a fuller description. ' The peribuccal ring,' according to Cameron, ' is prominent but is notched only laterally to give the appearance of single dorso-ventral teeth. Each tooth is complete and is *not* indented.' The

figure given of the ventral view of the head, though on a small scale, appears to show a slight indentation of the anterior margin of the ventral tooth. In any case, as will be seen below, the form of the circumoral ring and that of its processes are of little value as specific characters in '*S. labiato-papillosa*.' Cameron says of the spicules of the male that 'the smaller one is about 0·075 mm. long. The larger one is partly tubular and partly filamentous, the tubular part measuring 0·18 mm. long.' The size of the shorter spicule is certainly rather small for '*S. labiato-papillosa*,' but both spicules, judging from the figure, resemble those of the common species in form, and the length of the tubular, proximal part of the longer spicule is within the limits of variation. The length of the oesophagus ('0·35 mm.' [presumably 3·5 mm.] in the male and 4·56 mm. in the female) is rather less than is usual, but the rest of the measurements, and the other morphological characters described, do not appear to offer any bar to the inclusion of Cameron's material in the species '*S. labiato-papillosa*.' The writer, therefore, considers *S. bidentata* (Molin) a synonym of that species.

2. *Filaria digitata* v. Linstow, 1906. This form, originally described from *Bos indicus* in Ceylon, was referred by Railliet and Henry (1911) to the genus *Setaria*. Boulenger (1921) assigned some female specimens from Burmese domestic cattle to this species, and gave a description of them. He considered the smooth knob in which the tail terminated, and certain very slight differences in the shape of the cuticular ring surrounding the mouth, sufficient characters to distinguish the species from '*S. labiato-papillosa*.' Purvis (1931), however, has examined a large amount of material from buffaloes and cattle in Malaya, and finds that the form of the circumoral ring and of its processes is so variable as to be quite unreliable as a specific character, while the presence or absence of spines on the terminal knob of the tail in the female is also a very uncertain criterion. Purvis says: 'I have found the terminal knob of *digitata* to be often absent, while the spines of *labiato-papillosa* vary from being absent, to a termination showing irregularity of outline, to small spines—some large and some small, right up to the stage figured by Boulenger [(1921)] where the spines are fairly numerous and fairly large.' Taking other characters into consideration, and having found it impossible, on the basis of any of them, to group his material into two species, Purvis concludes that *S. digitata* is a synonym of *S. labiato-papillosa*.

Through the kindness of the Director of the Colombo Museum, the writer has had the opportunity of re-examining v. Linstow's original specimens of *S. digitata*. The shape of the circumoral ring is of no value in this case, since in almost every specimen it has evidently been damaged in places—and here it may be remarked that the writer has found this to be very frequently the case in *Setaria*, a fact which must be taken into consideration before basing specific distinctions upon this character. The tails of seven females were examined, and, while none of them had large spines, the terminal knob could in no case be described as perfectly smooth, but always showed some trace of wrinkling or 'shagreening.' v. Linstow gives the lengths of the spicules of the male as 0·16 mm. and 0·065 mm. respectively. The writer examined the spicules of the two males among the type material, and found that the shorter (right) spicule measured about 0·13–0·14 mm. It was not possible to determine the exact length of the longer (left) spicule, but it was apparently of the same shape as figured by Boulenger (1921), Thwaite (1927), Rajewsky (1928), Purvis (1931) and other authors for '*S. labiato-papillosa*,' and its length in both specimens was certainly over 0·3 mm., and in one apparently over 0·4 mm. v. Linstow's figure (1906, Pl. III, fig. 53) suggests that he mistook part of the right spicule for the shorter spicule, and did not observe the long left spicule at all. Accordingly, the writer has no hesitation in endorsing the view of Purvis that *S. digitata* is a synonym of '*S. labiato-papillosa*.'

3. *Setaria marshalli* Boulenger, 1921. This species was erected by Boulenger for a single female, in poor condition, found among his specimens of '*S. digitata*' from Burmese cattle. The 'lip-like lateral projections' of the circumoral ring were in this specimen 'exceptionally prominent and markedly tooth-like, being themselves indented at their apices,' while the lateral appendages of the tail were 'bifurcated and situated very close to the posterior extremity of the body.' The figure of the head suggests the retracted condition sometimes seen in '*S. labiato-papillosa*,' and the unusual shape of the circumoral ring might possibly be due to damage. It is less easy to account for the form of the tail, which is figured as being without a terminal knob, but having a pair of appendages, each with two angular points, situated at its extreme tip. While having a strong suspicion that this

was merely a shrunken and damaged specimen of '*S. labiato-papillosa*,' the writer feels that for the present the question must remain open.

4. *Setaria nudicauda* Ortlepp, 1924. This species was based by Ortlepp (1924) on a single female specimen from ' some species of deer ' in Dutch Guiana. The measurements of this specimen fall within the limits of variation for '*S. labiato-papillosa*,' except that the oesophagus is unusually short (about 3·9 mm.) and the nerve-ring and vulva rather nearer than usual to the anterior end. The specimen does not, however, appear to have been mature (' no mature eggs were present '). Concerning the armature of the mouth, Ortlepp says : ' The peri-buccal cuticular ring is unfortunately incomplete, its ventral and lateral parts being absent. The remaining dorsal prominence of this peri-buccal ring is simple and not notched.' The tail ' terminates in an obtuse tip,' no knob being shown in the figure, and the lateral processes are ' papilla-like,' apparently rather blunt, and situated very near the tip. In view of what has already been said as to variation in these characters, it seems doubtful whether they are sufficient to separate the species from '*S. labiato-papillosa*,' of which it is, in the writer's opinion, probably a synonym.

5. *Setara altaica* Rajewsky, 1928. This form, described by Rajewsky (1928, 1929) from *Cervus canadensis asiaticus* in Siberia, differs in no important respect, so far as the writer is able to discover from the description, from '*S. labiato-papillosa*.' Rajewsky states that it differs from all other described species of the genus in having five pairs of cephalic papillae, but this is the number usually observed in *S. labiato-papillosa* (in which Rajewsky describes seven pairs) and other species of *Setaria*. The morphology and measurements of the species show no difference sufficient to warrant its exclusion from the synonymy of '*S. labiato-papillosa*.'

6. *Setaria cervi* Maplestone, 1931. This form was described by Maplestone (1931) as a new species from *Cervus axis* in the Zoological Gardens, Calcutta, his description being based on two females only. Maplestone says that 'the worms are similar to *Setaria digitata*, according to the description of this species by Boulenger (1920) [i.e. 1921], in all their dimensions but they are a little smaller.' He states, however, that the ' chitinous mouth collar ' is much more prominent, ' as it projects about 0·22 mm. beyond the anterior end of the worm, and within it there is a definite cuticle-lined mouth of a corresponding depth, whereas the mouth collar of *S. digitata* appears to be only about 0·009 mm. in length, from Boulenger's figure.' It is rather difficult to compare the figures of the head given by Boulenger and by Maplestone, or to determine exactly at what point the latter has taken his measurements, but judging from the stated magnification of Boulenger's figures the height of the circumoral ring at its most prominent part appears to be about 0·023 mm. (not 0·009 mm.), while from the scales attached to Maplestone's figures it seems to be not much more than 0·025 mm. (not 0·22 mm.). This supposed difference between the species therefore disappears. Other differences mentioned by Maplestone are that his worms lack the lateral projections of the ' mouth collar ' described by Boulenger, and that the tip of the tail is more obtuse and the lateral processes nearer to it than in *S. digitata*. In view of what has already been said concerning the variability of these characters, the writer has no hesitation in regarding *S. cervi* Maplestone as a synonym of '*S. labiato-papillosa*.'

7. *Setaria buxi* Bhalerao, 1933. This species is based on a single female recorded by Bhalerao (1933) from a ' hill goat ' or Himalayan ibex (*Capra sibirica*) at Muktesar, United Provinces, India. Although all the measurements given are rather below the average, the specimen was, in the writer's opinion, probably referable to '*S. labiato-papillosa*.' The circumoral ring is described and figured as deeply notched laterally, with tooth-like processes projecting into the notches from the dorsal and ventral prominences. The latter are said not to be notched as in '*S. labiato-papillosa*.' The tail ends in an irregular knob bearing on one side a well-developed spine. These slight peculiarities, however, do not appear to be outside the possible range of variation for the common species.

To sum up what has been said, it is suggested that the name *Setaria cervi* should replace *Setaria labiato-papillosa*, and the following provisional, and probably incomplete, synonymy may be given :—

Setaria cervi (Rud., 1819)

Synonyms :—

Filaria papillosa auctt. (part).

Filaria oculi auctt. (part).

Filaria cervi Rudolphi, 1819. *Entozoorum Synopsis*, p. 8.

Filaria cervina Dujardin, 1845. *Hist. Nat. des Helminthes*, p. 49.

Filaria terebra Diesing, 1851 (part). *Syst. Helm.*, **2**, 274.

Filaria bidentata Molin, 1858. *Sitz. K. Akad. Wiss.*, Wien, **28**, 401.

Filaria terebra Dies., of Molin, 1858. *Ibid.*, 405.

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'MOSSY' FOOT IN NORTHERN ETHIOPIA

BY

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(*Received for publication* 6 *July*, 1936)

Recently, whilst serving with the British Red Cross Unit (British Ambulance Service) in Ethiopia, we encountered at Waldia numerous cases of a condition resembling that described by Thomas in 1910 as 'mossy' foot. Waldia is a small town situated about 75 miles north of Dessie, at an altitude of some 5,000–6,000 feet. We had gone there in response to an urgent appeal for medical aid after the bombardment of the town by Italian aeroplanes on January 15th, 1936. After the first rush of wounded had been dealt with, the peoples of the surrounding country began to bring to our camp for treatment medical and surgical cases of all descriptions, until at last we were treating in the out-patient section upwards of 100 new cases daily. Amongst the first 1,000 of these were 31 suffering from this condition resembling 'mossy' foot.

Of these 31 patients it was possible to keep some sort of record. Twenty-four were men and seven women; but, as the majority of the patients who came for treatment were men, these proportions are probably without significance. The duration of the disease varied from 1 to 20 years, but was usually long, the average being 6 years. Broadly speaking, the longer the duration the more advanced the disease. The ages of the patients varied from 10 to 60 years, and averaged 37 years.

The condition usually affected both feet. It appears to start as a firm oedema, affecting especially the dorsal surface. The soles are not affected even in advanced cases. Soon after the appearance of the oedema, the skin at the bases of the toes begins to show a papillomatous roughness, especially in the middle of the foot, which is at first velvety, and progressively spreads and coarsens until the whole dorsal surface of the foot, and the sides, may be covered by a dense mass of warty outgrowths. Sometimes cracks develop in the thickened skin, and occasionally septic wounds; but usually such complications are lacking. The oedema may reach well up the leg in advanced cases. The skin itself is enormously thickened. In one case, on which a biopsy was performed by Dr. C. E. Bevan to remove a piece for microscopical examination, the skin on the dorsum of the foot at the bases of the toes was found to be about half an inch in thickness. Apart from the swelling of the feet and the papillomatous growths, the condition apparently causes little trouble. Some patients complain of pain in the feet, but this is not invariable. The papillae are not tender. Mobility is not affected, the joints are not affected, and sinus formation such as occurs in Madura foot does not take place. As has already been mentioned, the soles of the feet are not affected, the skin there remaining of the normal thickness and being as soft as usual, or perhaps a little softer. There is a

tendency for the toes to be spread out. This is well shown in the accompanying photograph (taken by Dr. C. A. V. Barkhuus), which, however, was taken at too great a distance to show clearly the ' mossy ' condition.

As to the cause which leads to the development of the condition, no information was obtained from the patients, who invariably stated that the disease had developed ' of itself ' and had slowly progressed. No history of injury was elicited, and no parasitic infections to account for it were discovered. Filariasis occurred at Waldia, but was not especially common.

Loewenthal (1934) has recently summarized the literature on ' mossy ' foot, and has suggested that several diseases may be included in the category. The cases seen at Waldia clearly belong to the type for which he proposes the name ' lymphostatic verrucosis.' This disease has previously been recorded from several parts of Africa. The object in writing this note is merely to call attention to the apparent abundance of cases in a particular locality in northern Ethiopia. Unfortunately the conditions under which we worked were not favourable for any detailed study, and we are not able to throw any light on the causation and treatment of the disease.

REFERENCE

LOEWENTHAL, L. J. A. (1934). On the probable inclusion of several diseases in the title ' " mossy " foot.' *Ann. Trop. Med. & Parasitol.*, **28**, 47.

PLATE X

EXPLANATION OF PLATE X

A case of ' mossy ' foot in northern Ethiopia, showing the tendency of the toes to spread out.



H. R. Grubb, Ltd., Poplar Walk, Croydon.

A NOTE ON THE PREVALENCE OF DISEASE IN NORTHERN ETHIOPIA

BY

J. W. S. MACFIE

(*Received for publication* 6 *July*, 1936)

An opportunity occurred recently, whilst I was serving with the British Red Cross Unit in Ethiopia, of examining a large number of patients at Waldia, a locality which had not previously had the benefit of a medical service. (Waldia is about 75 miles north of Dessie, and stands at an altitude of 5,000-6,000 feet. The surrounding country is mainly mountainous. The climate is apt to be damp, and is cold at night.) Our Unit had gone to Waldia to attend to the wounded after the bombardment of the town by Italian aeroplanes on January 15th, 1936, and was held up there for over 5 weeks, awaiting the completion of the road northwards to Kworam. During this period patients from the surrounding country poured in in embarrassing numbers, our Unit being equipped primarily for the treatment of wounded, and not especially for dealing with medical cases. The subjoined list may be of interest as a sample, indicating the prevalence of various diseases in this part of Ethiopia. The diagnoses were necessarily perfunctory in many cases, but, such as it is, the list shows the complaints of all the (989) patients who came for treatment to our out-patient tent during the 12 days, January 21st to February 1st, 1936. The cases were consecutive, and were not selected in any way, excepting only that after 133 cases of tape-worm infestation had been treated our supply of the appropriate medicines was temporarily exhausted and, therefore, no more of these could be attended.

ANALYSIS OF 989 CONSECUTIVE UNSELECTED CASES TREATED AT WALDIA, JANUARY 21ST TO FEBRUARY 1ST, 1936

Minor injuries, abscesses, ulcers, boils, septic sores, etc.	209	Anaemia, debility, etc.	7
Tape-worm infestation	133	Dysentery (amoebic)...	7
Rheumatism, myalgia, lumbago, etc.	100	Nasal conditions : discharge, etc. ...	6
Syphilis	89	Pharyngitis	6
Dyspepsia, gastric catarrh	52	Leprosy	4
Eye conditions (mostly conjunctivitis)	50	Elephantiasis : leg, scrotum ...	4
Scabies	47	Lipoma	3
' Mossy ' foot	29	Hernia : inguinal	2
Cough, bronchial catarrh, bronchitis, etc.	28	Orchitis	2
Gonorrhoea	25	Glossitis	2
Impetigo	24	Hare lip	2
Malaria	20	Prostatic enlargement	2
Teeth, carious	20	Mollusca fibrosum	2
Diarrhoea	17	Warts	2
Injuries by bomb, gun or sword ...	16	Goitre, Haemorrhoids, Appendix abscess, Ascites (hepatic), Tinea, Varicose veins, ' Fibroid,' Paralysis, Fistula, Stricture (urethral), Asthma, Hydrocoele, Epilepsy, Morbus cordis, Herpes labialis, Paralysis agitans, Influenza and Goundou, of each...	1
Ear conditions, mostly otitis ...	15		
Dermatitis, eczema, etc.	13		
Constipation	10		
Headache	8		
Tuberculosis : lung, glands, etc. ...	8		
Synovitis : knee, etc.	7		

Many of these cases were in a very advanced stage, and presented appearances such as nowadays are never seen in any civilized State. To judge by the reactions of our warrant officers, the tertiary syphilides were the most remarkable. Some of the prodigious gummata were indeed impressive, and satisfactory, in that they responded to treatment very readily. There is at Dessie, 75 miles further south, a well-established and widely appreciated Medical Mission which has for years done valuable work on behalf of the health of the natives of Ethiopia. It was probably the echo of the good report of this work which, reverberating amongst the mountains at Waldia, accounted for the fact that many of our patients clamoured for ' injections,' insisting, often quite untruly, that they had syphilis.

Tape-worm infestation is almost an institution, raw meat being a favourite article of diet, and the drinking of ' koso,' the native medicine for it, a periodical social, if not also religious, rite. The people certainly know all about the symptoms and signs produced by the worm, and appreciated the fact that our ' koso,' male fern and no. 9 pill, was better, and less dangerous to take, than their own. So popular was the remedy, indeed, that if for any reason money was unsuitable, a dose of ' koso ' would serve as a small reward or tip. For as long as we could, we treated about 30 cases daily. But officers of the Imperial Guard soon showed an inclination to send us whole companies of their men for treatment, and our resources would not stand that !

Two cases of favus, concealed in the list, interested me because I do not recollect having seen this disease in West Africa. Another contrast with West African experience was the relative infrequency of hernia. This is to be accounted for, perhaps, by the fact that Ethiopians at Waldia do not from childhood carry heavy loads on their heads, as do West Africans. Hydrocoele was also much less common than it is in West Africa.

One typical case of Madura foot was treated. ' Mossy ' foot, or rather that condition resembling it for which Loewenthal (1934) has proposed the name ' lymphostatic verrucosis,' was common, and is the subject of a separate note (see p. 299).

All the dysentery cases seen were amoebic. The malaria infections, when confirmed by microscopical examination, were sub-tertian. The case recorded as goundou was an adult man who showed all the usual appearances, as well as a swelling on the forehead at the base of the nose. Typhus and relapsing fevers, which do not appear in the list, were in fact probably rather common.

REFERENCE

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS GLOSSINA WIEDEMANN BASED ON A COMPARATIVE STUDY OF THE MALE AND FEMALE TERMINALIA

(*Continued from page* 168)

BY

W. S. PATTON

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication* 10 *July*, 1936)

Glossina pallidipes Austen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. Very similar to, but distinctly larger than, most specimens of *morsitans*. Third segment of antenna (fig. 1) narrower, longer, more pointed and more bent forward at the end than that of *morsitans*, the anterior border with some fairly long hairs. Tarsi of fore and mid legs yellow, last two segments of hind tarsi brown. Dark bands on abdominal terga darker, wider and much better defined than in *morsitans*. DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci*. Fig. 2, *b*, *c*. Each cercus is narrower than that of *morsitans*, and the two are united together distally by lighter chitin, the remainder by a strong spinose membrane. The external distal border ends

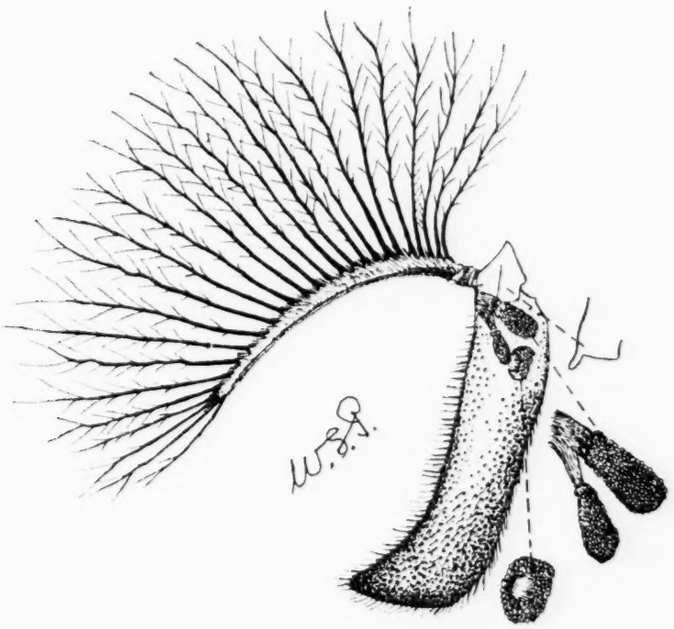


FIG. 1. Antenna of ♂ *pallidipes* ; peg and sensoria shown enlarged ; note the pointed end of the third segment of the antenna and the rather long hairs along its anterior border.

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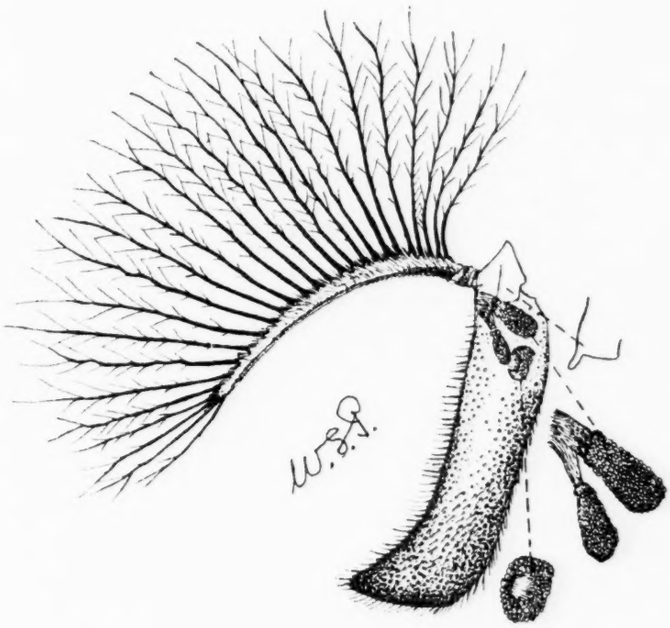


FIG. 1. Antenna of ♂ *pallidipes* ; peg and sensoria shown enlarged ; note the pointed end of the third segment of the antenna and the rather long hairs along its anterior border.

in a stout rounded projection. Each cercus bears a number of long, stout hairs on the outer border, mostly attached on the dorsal side. There is the usual row of short, stiff, pointed hairs extending round the distal margin internally and dorsal to the membrane. *Phallosome*. Fig. 3, *a*. Very similar to that of *morsitans*, with the usual finger-like spinose membrane at the junction of the apodeme with the ninth tergo-sternum. *Paramere*. Fig. 3, *b*. Similar to that of *morsitans*, but larger and with numerous long hairs on the outer side. ♀. Fig. 3, *e*. The

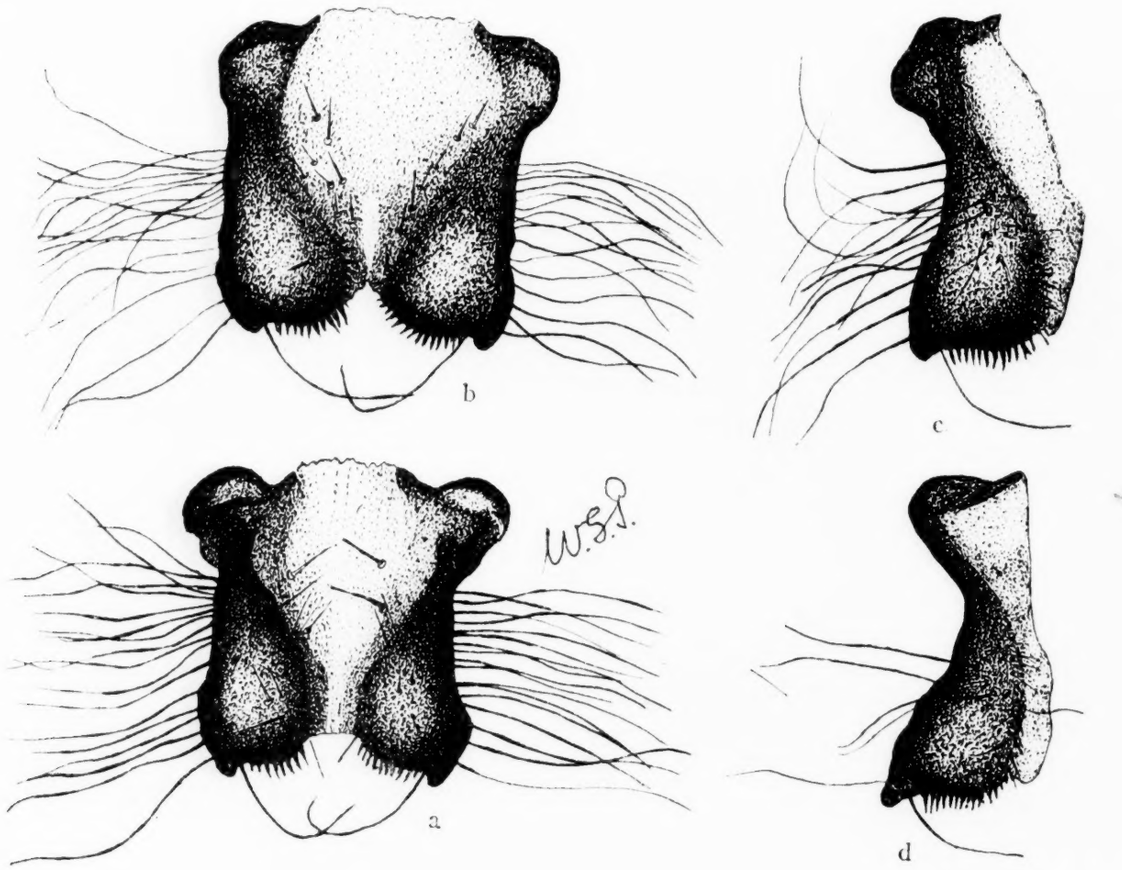


FIG. 2. *a*.—Ventral view of anal cerci of *longipalpis* ; *b*.—Ventral view of anal cerci of *pallidipes* ; *c*.—Dorsal view of one anal cercus of same ; *d*.—Dorsal view of one anal cercus of *longipalpis*.

ninth tergum consists of two separate oval plates joined by incomplete chitin; otherwise the terminalia are similar to those of *morsitans*.

DISTRIBUTION. HABITS. IMPORTANCE. *G. pallidipes* is found only in East Africa. It has a wide distribution, extending northwards into Abyssinia and Italian Somaliland (about 6° N.); it is found in the southern and western parts of Uganda, the Semliki Valley, around Lake Albert and the northern shores of Lake Victoria, throughout Kenya and northern Tanganyika, Rhodesia and Jubaland. It is a very active species and will readily bite at night. It can adapt itself to various types of country, woods or savannah, open forest scrub and thicket, and

is much more dependent on water than *morsitans*. Experimentally it is a good transmitter of pathogenic human trypanosomes, but as it is nearly always found in company with *morsitans* there is no definite proof that it transmits these parasites in nature. In Italian Somaliland it is said to transmit *T. brucei* to horses and cattle and *T. congolense* to cattle.

Glossina longipalpis Wiedemann. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A medium-sized to relatively large species, somewhat resembling *morsitans* var. *submorsitans*, with which it may be confused by the beginner. The third segment of antenna (fig. 4) is

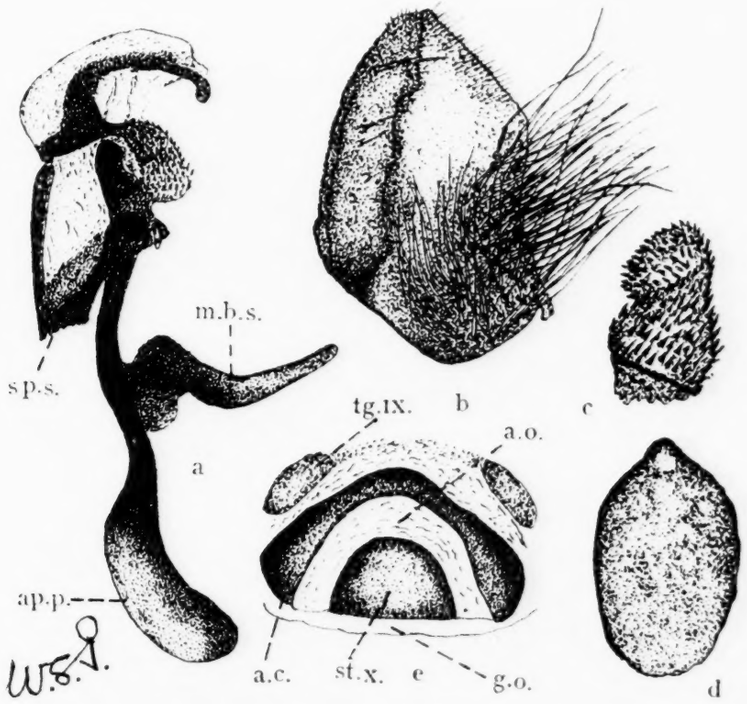


FIG. 3. *a*.—Phallosome of *pallidipes* dissected out of ninth tergo-sternum, a part of which is left; *ap.p.*—apodeme of phallosome; *m.b.s.*—median binding sclerite; *s.p.s.*—sperm pump sclerite; *b*.—Left paramere; *c*.—Spined finger-like membraneous process at junction of each side of ninth tergo-sternum and phallosome; *d*.—Plate on ventral side by which phallosome articulates with distal end of ninth tergo-sternum; *e*.—Terminalia of ♀; *a.c.*—anal cerci; *a.o.*—anal opening; *g.o.*—genital opening; *tg.ix.*—ninth tergal plates, which are incompletely developed; *st.x.*—Tenth sternum.

distinctly longer and has longer hairs along the anterior border than in *morsitans* var. *submorsitans*; this is a fairly reliable character, and can be made out with a good pocket-lens. The brown bands on the abdominal terga are usually not so well defined as in *morsitans* var. *submorsitans*; they vary in extent, and in some specimens are well marked on the distal segments. Legs marked as in *morsitans* var. *submorsitans*.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci*. Fig. 2, *a*. From a comparison of figs. 2, *a*, and 2, *b*, it will be noted that the anal cerci are very similar to those of *pallidipes*; they are, however, distinctly narrower, the

semi-membraneous chitin joining the two cerci being wider than in *pallidipes*. *Phallosome*. Fig. 5, *a*. The phallosome of *longipalpis* is very similar to that of *pallidipes*, and, like it, has no fine hairs on the sides of the distal rounded end. *Paramere*. Fig. 5, *b*. The paramere is very similar to that of *pallidipes*, and also has very long hairs on the outer side in the hollow. ♀. Fig. 5, *e*. The ninth tergum consists of small lightly chitinized plates, as in the ♀ *pallidipes*.

DISTRIBUTION. HABITS. IMPORTANCE. *G. longipalpis* is mainly limited to West Africa, where it is widely distributed in Sierra Leone, Gold Coast, Ivory Coast, French Guinea, Senegal, Northern and Southern Nigeria and North Angola. It has been recorded from the Katanga district of the Belgian Congo at Lakago, and is probably to be found in small numbers throughout the southern

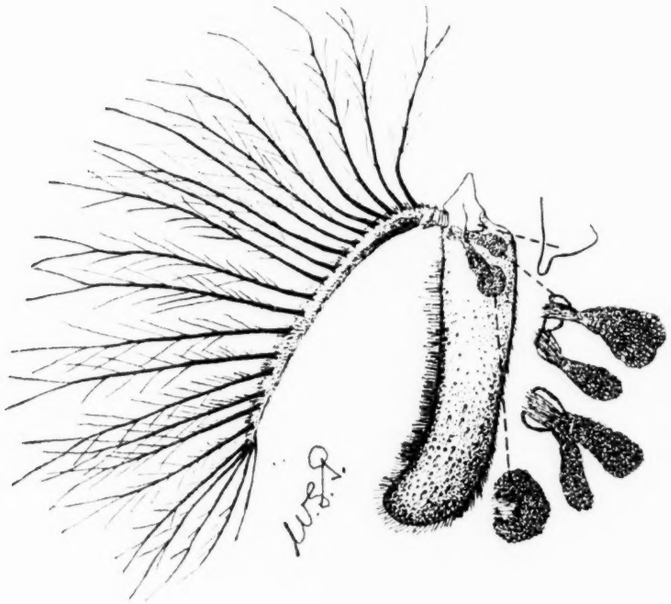


FIG. 4. Antenna of ♂ *longipalpis* ; peg and sensoria shown enlarged ; note the end of the blunt third segment and the rather long hairs along its anterior border.

and western parts of the Belgian Congo. It is a species which can adapt itself to varying conditions of shade and humidity. It is generally found in open country with grass and thickets, and not very close to water ; it has also been recorded at the edges of dense forests in the vicinity of water. Morris (1934) has recently made an exhaustive study of the bionomics of *longipalpis* in the Gold Coast. He shows that in the Gold Coast the distribution of *longipalpis* is dependent on the humidity of the ecoclimate rather than on temperature, and that it is mainly found in transitional forest, inland savannah and coastal savannah ; it does not occur in the rain forest. The main breeding season is from March to July. It is an important transmitter of *T. gambiense*, *T. congolense* and *T. vivax*.

Glossina austeni Newstead. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A small slender species, with a characteristic reddish abdomen ; it is an exception in this group, in that the hind tarsi are all dark, like those of the species of the *palpalis* group. The third segment of the antenna (fig. 6) is rounded at the end. The abdominal markings vary, the dorsal surface usually being of a bright reddish or reddish-yellow colour, and the dark markings on the terga generally consisting of brown blotches ; in some specimens, however, they form more or less interrupted bands.

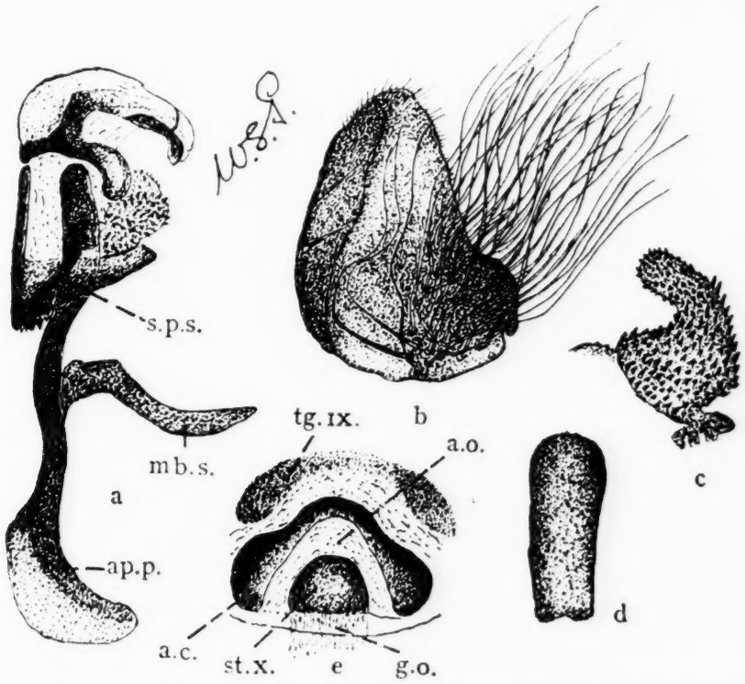


FIG. 5. *a*.—Phallosome of *longipalpis* dissected out of ninth tergo-sternum ; lettering as in fig. 3, *a* ; the phallosome is very similar to that of *pallidipes* and, like it, has no hairs at the side ; *b*.—Left paramere ; *c*.—Spined finger-like membraneous process ; *d*.—Plate on ventral side by which phallosome articulates with ninth tergo-sternum ; note that it is narrower than that of *pallipides* (fig. 3, *d*) ; *e*.—Terminalia of ♀ ; lettering as in fig. 3, *e* ; they are very similar to those of *pallidipes*.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Fig. 7, *c*, *d*. The anal cerci are similar to those of *pallidipes* and *longipalpis* rather than to those of *morsitans* and its close ally *swynnertoni*. Each cercus is, however, more lightly chitinized, and the outer distal end does not terminate in such a dense tooth-like process as in *pallidipes* and *longipalpis*. The two cerci are joined in the middle line by a lightly chitinized membrane which projects beyond the end before joining with that of the other side, as shown in fig. 7, *c*. *Phallosome.* Fig. 7, *a*. Here again the phallosome is very like that of the other species of the group, and is linked to that of *morsitans* by the presence of fine hairs at the rounded end on each side. *Paramere.* Fig. 7, *b*. The paramere is a rather small plate, and has only a relatively few short hairs. ♀. Fig. 7, *e*. The ninth

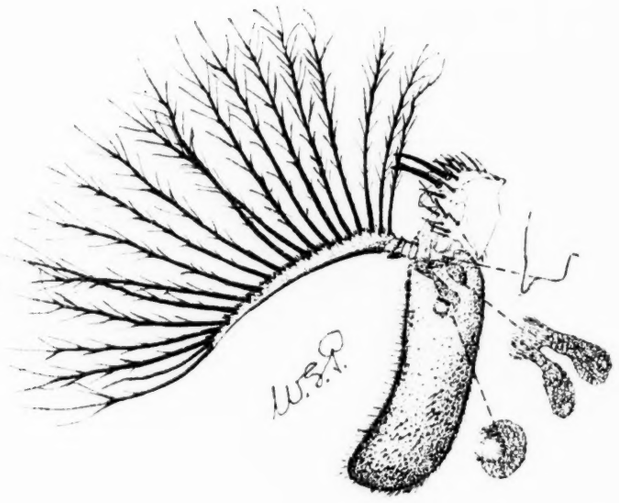


FIG. 6. Antenna of ♂ *austeni* ; peg and sensoria shown enlarged.

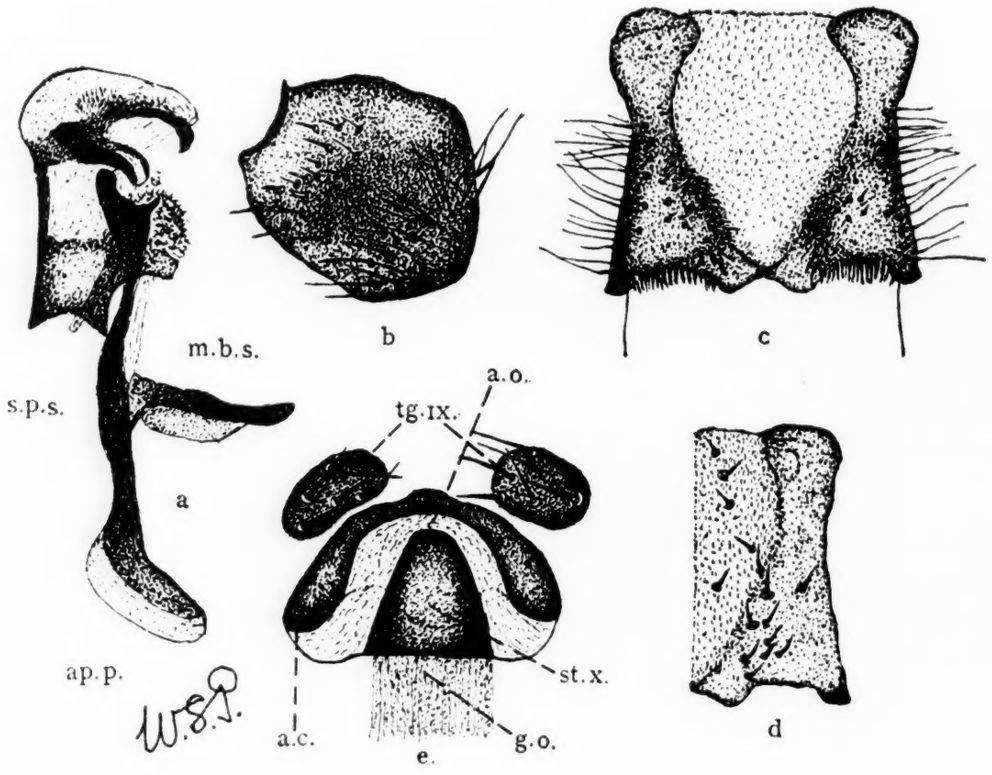


FIG. 7. *a*.—Phallosome of *austeni* dissected out of ninth tergo-sternum ; lettering as in fig. 3, *a* ; the phallosome is very similar to that of the other species of the group, and has hairs on the side of the phallosome, as has *morsitans* and *swynnertoni* ; *b*.—Left paramere ; it is wider but shorter than that of either *pallipides* or *longipalpis*, and the hairs are shorter ; *c*.—Ventral view of anal cerci ; note how they are joined in the middle line distally ; *d*.—Dorsal view of one anal cercus ; *e*.—Terminalia of ♀ ; note the large separate spined tergal plates ; lettering as in fig. 3, *e*.

tergum consists of two relatively large and strongly chitinized, rounded or oval plates, covered with several strong bristles ; otherwise the terminalia are very similar to those of the other species. I think that there is very little doubt that *austeni* is a link between the *pallidipes-longipalpis* sub-group and the sub-group *morsitans-swynnertoni*.

DISTRIBUTION. HABITS. IMPORTANCE. *Glossina austeni* is said to extend over a large part of East Africa, from Jubaland to Zululand in the south. It is found in many localities in Kenya, Portuguese East Africa, Tanganyika and Zululand. It is usually encountered in dense vegetation and among thickets not very far from water. Nothing is known of its importance as a transmitter of trypanosomes.

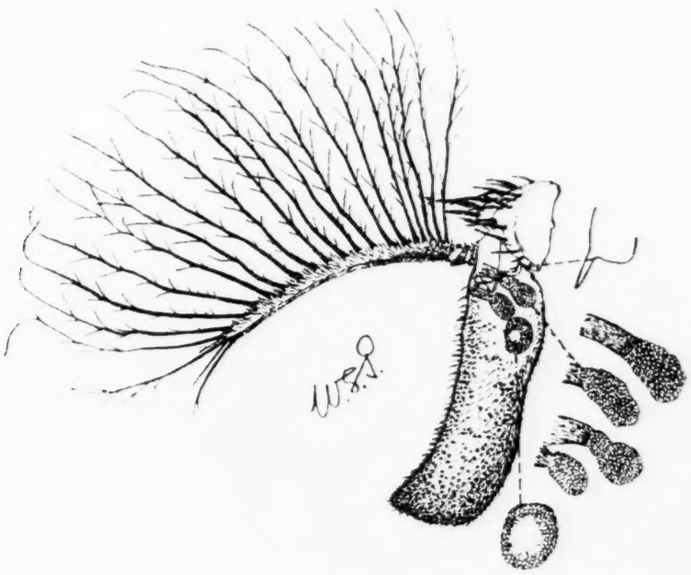


FIG. 8. Antenna of ♂ *palpalis*, western form ; peg and sensoria shown enlarged.

SPECIES OF THE PALPALIS GROUP

Before noting the main diagnostic characters of the species of this group, it is necessary to point out that the technique employed in mounting the ♂ terminalia is of the first importance. In the past it has been the custom to mount the entire terminalia of the ♂ *Glossina* by flattening them out under a coverslip (after clearing, etc.) and extending the anal cerci. In such a preparation the phallosome is not only obscured but is flattened in the dorso-ventral position, and as a result a good character on it has been overlooked. The anal cerci, too, in such a preparation are distorted, and the parameres are often flattened. In order to study the parts and to gain a correct idea of their structure, it is necessary to mount them after dissection in Canada balsam on slides without compression, and to orientate them all in the same way so as to get uniform results when illustrating them.

It will be remembered that the anal cerci in this group consist of a pair of plates which end distally on the outer side in a short (sometimes long) tooth-like process, and that the two cerci are joined by a membrane which does not extend to the end of the process, and is deeply incised in the middle ; and as the membrane is very loose the cerci can be widely extended (fig. 9, *j*). The length of the

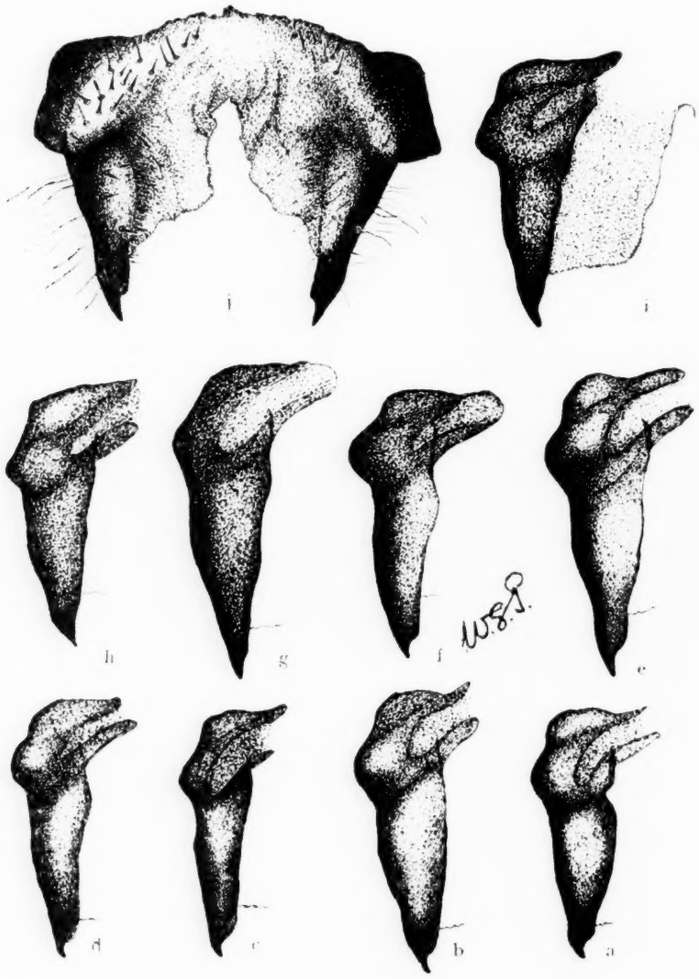


FIG. 9. *a*.—Ventral view of left anal cercus of *palpalis* var. *fuscipes* from Unyoro district, Uganda ; *b*.—Same from a specimen from Lusamu, Zambezi ; *c*.—Same from a specimen from Damba Island, Lake Victoria ; *d*.—Same from a specimen from Olwal, Uganda ; *e*.—Same from a specimen from Montwadi Island, Lake Tanganyika ; *f*.—Same of a specimen of *palpalis* type form from Kwango, Congo-Angola frontier ; *g*.—Same of a specimen from the same locality ; *h*.—Same of a specimen of *palpalis* var. *wellmani* (western form) from Ossidingi, S. Nigeria ; *i*.—Same of a specimen from Sierra Leone ; *j*.—Ventral view of anal cerci of type form from the Lomami District, Belgian Congo.

tooth-like process and the presence of a shelf on its inner side near the end are points of diagnostic importance. The phallosome is structurally similar to that of the *morsitans* group, and is embedded in the same way in the ninth tergosternum (fig. 10, *a*). The apodeme, as in all the species of *Glossina*, ends in a keel-like plate, the shaft forks distally into two limbs, united by chitin, against

the end of each of which the phallosome proper is hinged. At this point on each side there is (in all the species of *Glossina*) a spinose, finger-like process which varies in length in the species (fig. 10, *a*, *c*). It is illustrated in many of the drawings in these papers. At the distal end of the apodeme in the middle line on the dorsal side there is a characteristic hollow hook-like process (fig. 10, *f*), in the ventral cavity of which the head of the long sperm pump sclerite

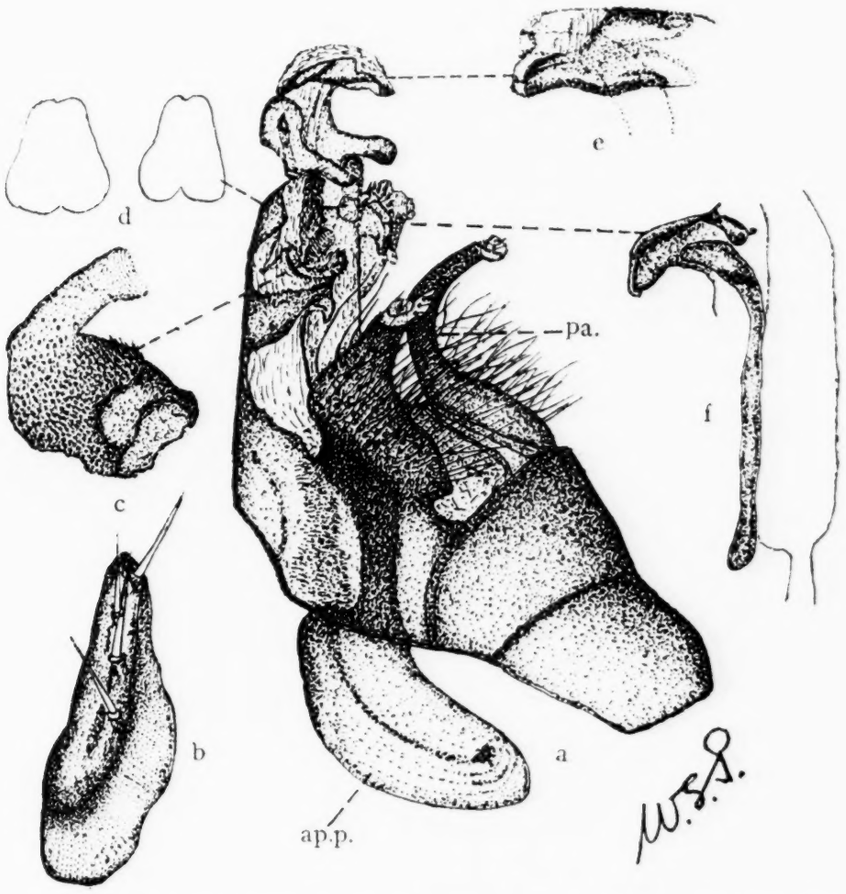


FIG. 10. *a*.—Ninth tergo-sternum of *palpalis*, western form, showing the phallosome embedded in its dorsal concavity ; note the characteristic shape of the parameres when seen in side view ; *ap.p.*—apodeme of phallosome ; *pa.*—paramere ; *b*.—End of paramere enlarged ; *c*.—Spined finger-like membraneous process at junction of ninth tergo-sternum and phallosome ; *d*.—Plate on ventral side of phallosome showing variation ; *e*.—End of phallosome ; *f*.—Dorsal process of apodeme at distal end in the middle line, showing the head of the sperm pump sclerite lodged in its ventral hollow, and the dilated ejaculatory duct attached to the sclerite.

lies. The phallosome proper consists of a small chitinous framework carrying the ejaculatory duct, on each side of the opening of which there is a long chitinous (in side view) rod-like plate (fig. 10, *e*) ; these plates are continued backwards, and support the ventral membrane closing this part. The plates are fused with the rounded body of the phallosome, which is continued backwards and upwards as two diverging rods whose rounded ends articulate with the end of each fork of the apodeme (figs. 10, *a* ; 11, *a* ; 12, *a*). In the middle the two rods are

connected with a pair of foot-like processes, one on each side of the middle line, which articulate with the heart-shaped plate at the distal end of the ninth tergo-sternum on the posterior side ; this forms the posterior hinge for the phallosome. Between the two distal arms of the phallosome (which in side view form the rounded postero-ventral border), there is on each side of the middle line a pair of plates (or rods) which are continuous with the two diverging rods mentioned above (figs. 11, *b* ; 12, *b* ; 13, *b* ; 14, *c* ; 15, *a*) ; these two small plates can only be seen when the phallosome is mounted ventral surface uppermost ; if compressed dorso-ventrally under a coverslip, their structure cannot be satisfactorily made out.

The paramere in the *palpalis* group is a wide plate, convex externally and attached to the side of the ninth tergo-sternum (fig. 10, *a*) ; the inner margin is free and is extended into a long neck-like process terminating in an expanded plate, which bears a number of hairs (fig. 10, *a*). The external surface bears a number of very long hairs, and in addition there may be numerous fine microscopic hairs on its surface ; but these may be wanting. The outer margin of the paramere may be either uniformly sloped or raised into a characteristic shoulder. On the inner side, each paramere is loosely attached by membrane to the median binding sclerite. As the shoulder on the outer side of the paramere has been used as a character to define certain species, its shape is important and can only be satisfactorily studied when the paramere is dissected off and mounted without compression in Canada balsam. I have been able to make an extensive study of the structure of the phallosome, paramere, and anal cerci of specimens of *palpalis* from many localities throughout its extensive distribution, and as a result some new facts regarding this species and its varieties have come to light. No useful purpose would be served by describing the external characters of the adults of *G. palpalis*, except to note that, like most of the species of this group, there are no dark bands on the abdominal terga interrupted in the middle line. The colouring is either dark or very dark, or may be lighter especially towards the base of the abdomen. Then again there is the var. *wellmani*, in which the thoracic markings are much reduced ; there are also some characteristically small specimens, such as that recorded by Macfie from Ilorin, in which the abdominal markings are rather like those of *tachinoides*. As in all the species of this group, the hind tarsi are dark.

It will be recalled that the type of *palpalis* came from the Congo, the exact locality not being known. I have examined the terminalia of many specimens from the Congo collected by Dr. Schwetz, especially those from the Lomami district and along the Congo-Angola frontier, and have found a striking character on the phallosome which is peculiar to specimens from this part of the Congo. I, therefore, propose to regard this as the type form, and will shortly describe its terminalia and those of the two varieties which I recognize.

Glossina palpalis Robineau-Desvoidy (type form). DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Fig. 9, *f*, *g*, *j*. Outer tooth-like process

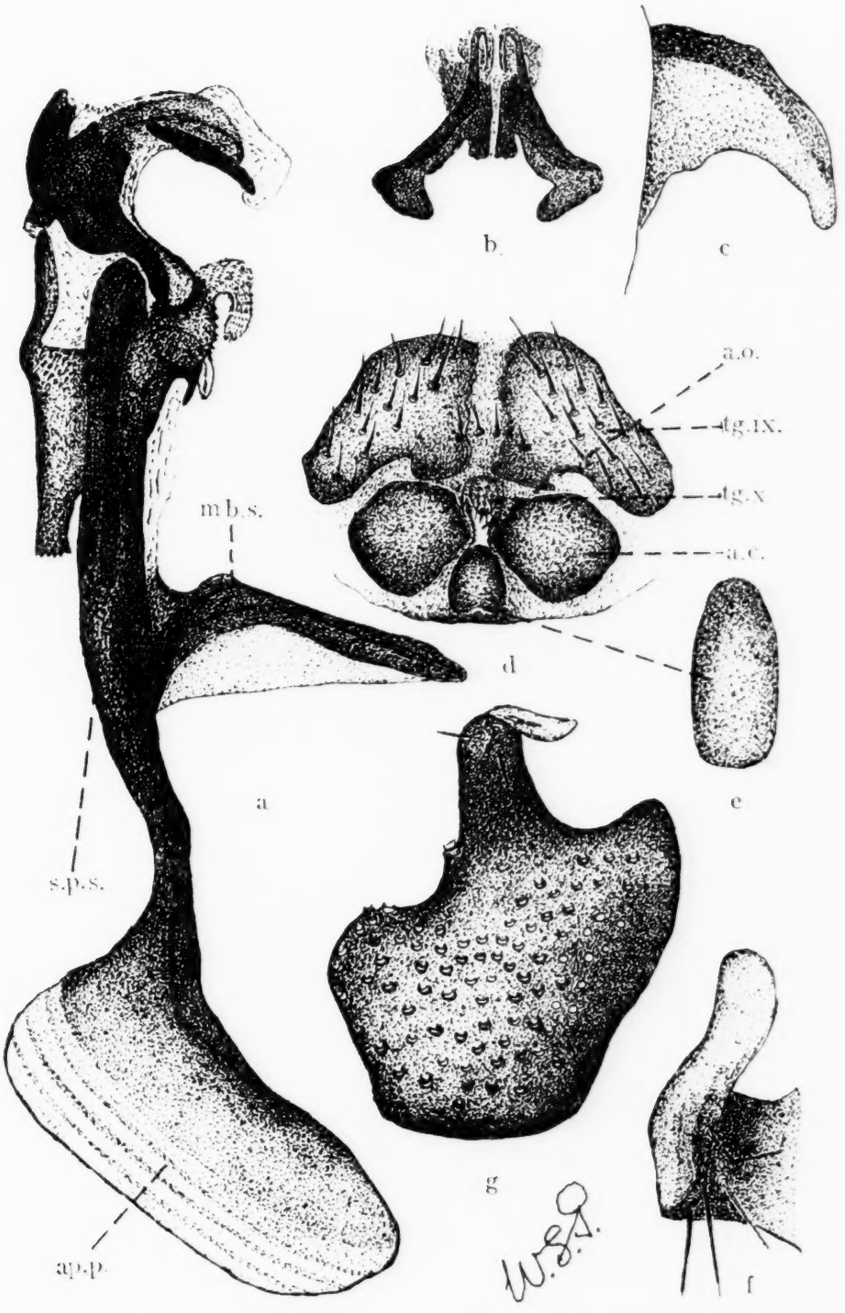


FIG. 11. *a*.—Phallosome of type form of *palpalis* from Lomami District of Belgian Congo ; note the two plates which project upwards on the ventral side of the rounded part of the phallosome, forming a partial hood ; lettering as in fig. 3, *a* ; *b*.—Enlarged ventral view of the two plates, showing their structure and how they originate ; *c*.—Hook-like dorsal projection at end of apodeme in the middle line ; *d*.—Terminalia of ♀ ; *tg.x*.—tenth tergum ; other lettering as in fig. 3, *e* ; *e*.—Tenth sternum of ♀ ; *f*.—End of right paramere ; *g*.—Left paramere ; note the bluntly pointed shoulder on the outer side.

fairly long and pointed, with a well-marked shelf on the inner side some distance from end (fig. 9, *j*). In specimens from the Congo-Angola frontier it was noted that the process may be very short, with a less marked shelf (fig. 9, *f*), or the shelf may be entirely wanting (fig. 9, *g*), as in the anal cercus of the western variety (fig. 9, *h*, *i*). *Phallosome*. Fig. 11, *a*. The two rod-like extensions of the lateral arms are well developed, and when seen in side view project beyond the postero-ventral border of the phallosome (fig. 11, *a*, *b*); fig. 11, *b*, shows the two rods when seen from the ventral side. This is a striking character, and as far as I know is peculiar to specimens from the northern, central and western parts of the Congo. In specimens from the Congo-Angola frontier the rods are less well developed, suggesting that this form is linked up with the western variety. *Paramere*. Fig. 11, *f*, *g*. The shoulder on the outer side of the paramere is well developed and may be either bluntly pointed, as in fig. 11, *g*, or more rounded; the neck-like extension is short, and the expanded end is rather narrow, usually with four or five hairs. The outer surface is covered with long hairs and the microscopic ones are usually wanting. ♀. Fig. 11, *d*. In this form of *palpalis* and varieties the ninth tergum consists of two large plates (as in all the species of this group) covered with numerous short bristles, with a few in the membrane between the plates. The tenth tergum is a small oval plate. The anal cerci are large convex plates lying on each side of the anal opening, and the tenth sternum is a long plate, part of which is tucked back and forms the posterior wall of the genital opening.

Glossina palpalis var. **fuscipes** Newstead (eastern form). DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci*. Fig. 9, *a*, *b*, *c*, *d*, *e*. Similar to that of the type form, but shelf on inner side near end not so wide, and tooth-like process, though usually smaller, varies considerably in length. *Phallosome*. Figs. 12, *a*; 13, *a*; 14, *a*. Also similar to that of the type form, but the two rod-like extensions distinctly smaller (figs. 12, *b*; 13, *b*, *f*; 14, *c*) and hardly projecting beyond the postero-ventral border of the phallosome (fig. 13, *a*). *Paramere*. Figs. 12, *c*, *e*; 13, *c*, *e*; 14, *d*, *e*, *f*. Very variable in structure, the neck-like extension being either short and broad (figs. 12, *e*; 14, *f*), or long and narrow (fig. 13, *c*, *e*); the shape of the outer shoulder also varies: it may be relatively short, and either pointed or rounded, or longer and again either rounded or pointed. The expanded end also varies in shape and size, and may be short and wide, or longer and narrower; there may be from 3 to 5 hairs. The outer surface of the paramere has numerous long hairs, and in addition there are always numerous microscopic ones on the inner surface and towards the middle. ♀. Figs. 12, *d*; 13, *d*; 14, *b*. The ♀ terminalia are very similar to those of the type form, as will be seen by comparing figs. 11, *d*; 13, *d*. The differences in the shape and size of the different plates seem to be those of degree rather than of kind. There may be a few small bristles in the membrane between the two parts of the ninth tergum, or these may be wanting.

It will be remembered that the variety *fuscipes* was described from specimens collected by Dr. Shircore from the Nile Province of Uganda. Although I have not myself been able to examine the terminalia of specimens from the type locality, I have studied many microscopic preparations of the terminalia from

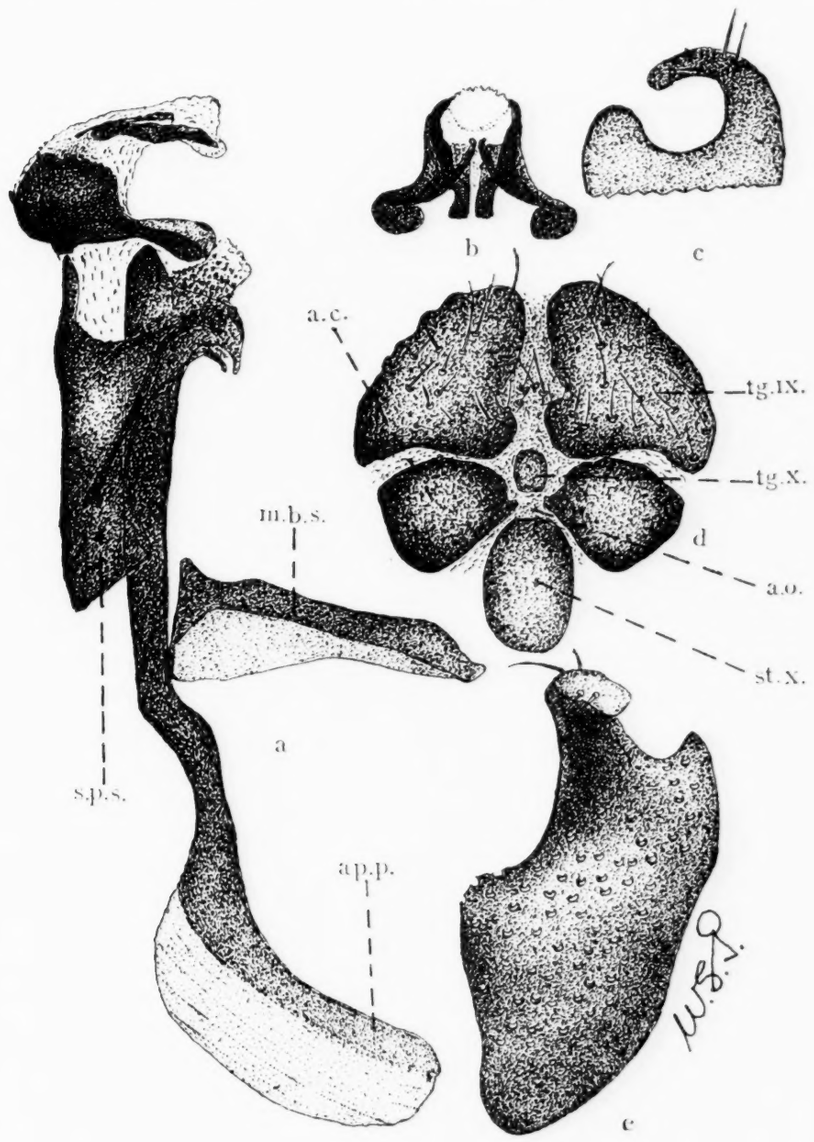


FIG. 12. *a.*—Phallosome of *palpalis* var. *fuscipes* from specimen from Damba Island, Lake Victoria ; *b.*—Enlarged ventral view of end of phallosome of same showing the small plates ; *c.*—End of right paramere of same showing somewhat rounded shoulder ; *d.*—Terminalia of ♀ ; lettering as before ; *e.*—Left paramere of same showing small pointed shoulder.

this locality. In addition, I have dissected and studied the terminalia of specimens from the Unyoro district of Uganda (fig. 13) and from Olwal and Toro (fig. 13) ; from Damba Island, Lake Victoria Nyanza (fig. 12) ; from Kenya, and from Lake Tanganyika (fig. 14). This study has convinced me that

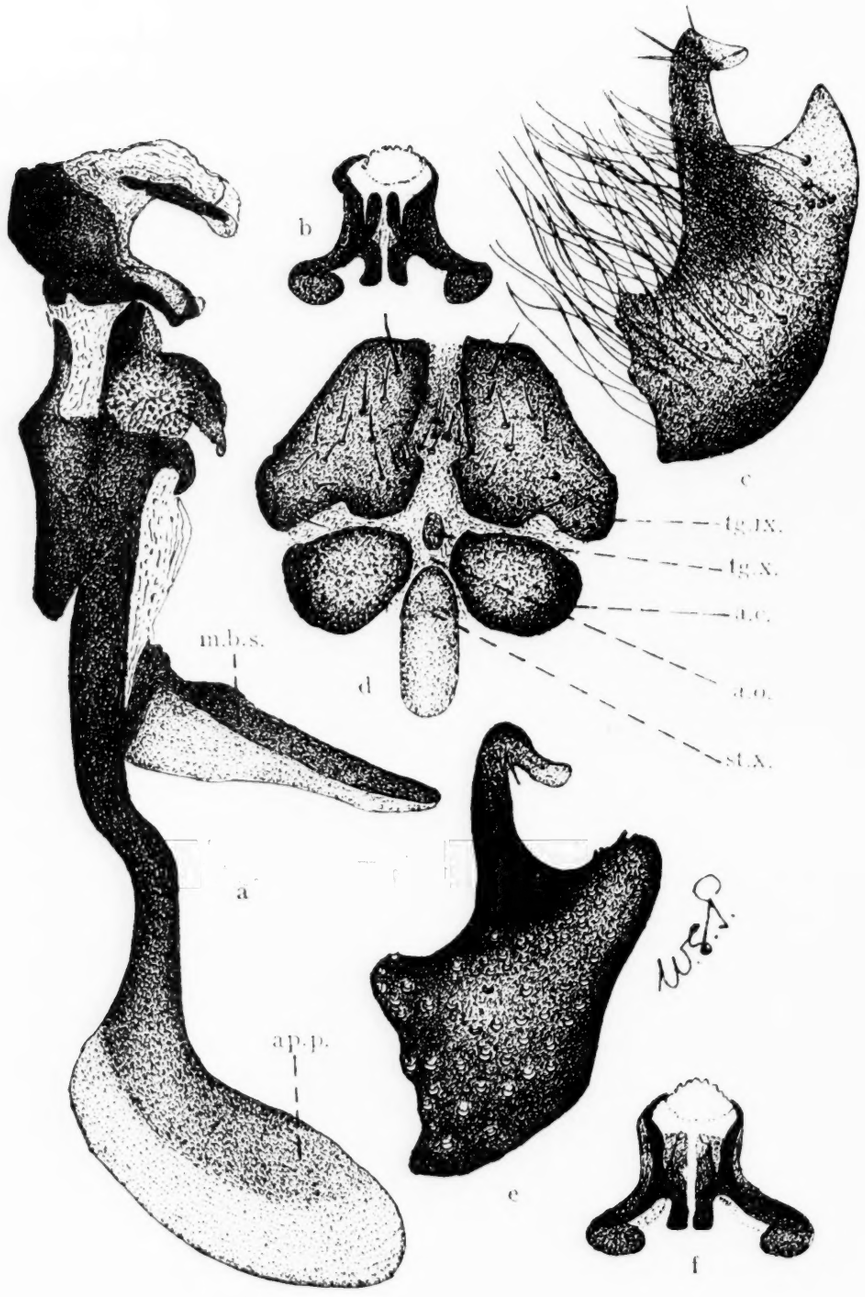


FIG. 13. *a.*—Phallosome of specimen of *palpalis* var. *fuscipes* from Unyoro district, Uganda ; the plates at the back of the phallosome are better developed than in the more southern form from Lake Tanganyika ; *b.*—Enlarged ventral view of phallosome showing the better developed plates ; *c.*—Left paramere of a specimen of *palpalis* var. *fuscipes* from Toro, Uganda ; note the pointed shoulder ; *d.*—Terminalia of ♀ of a specimen from Toro ; *e.*—Left paramere of specimen from Unyoro district, Uganda ; note the characteristic shape, the long neck and the narrow flat end; *f.*—Enlarged ventral view of end of phallosome showing the incompletely developed plates.

in *fuscipes* the structure of the distal end of the anal cerci, that of the paramere (particularly the neck-like process), the expanded distal end and the outer shoulder vary, not only in specimens from the same locality, but in those from the northern, central and southern parts of its distribution. In a recent paper, Zumpt (1935) has described the eastern variety of *palpalis* from Kasanga (Bismarckburg) as a new species, naming it *martinii*, and has raised *fuscipes* to

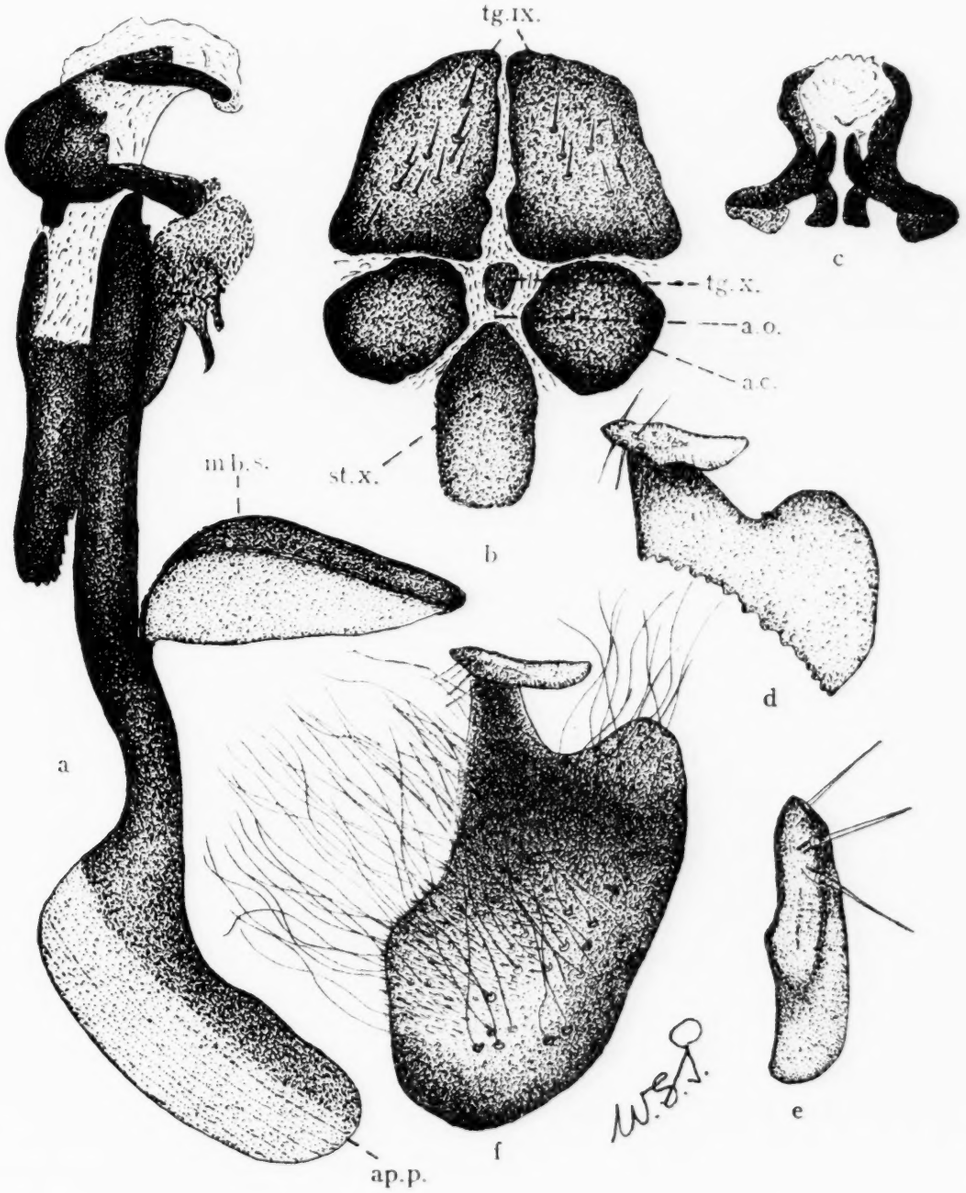


FIG. 14. *a*.—Phallosome of *palpalis* var. *fuscipes* from Montwadi Island, Lake Tanganyika ; the plates on the ventral side are small in this form and do not project when seen in side view ; *b*.—Terminalia of ♀ ; lettering as in fig. 3, *e*, and fig. 10, *d* ; *c*.—Enlarged ventral view of phallosome showing small plates which do not project ; *d*.—End of left paramere to show rounded shoulder ; *e*.—End of paramere showing bristles ; *f*.—Left paramere of another specimen from same locality ; note the more pointed shoulder ; all gradations between these two have been encountered.

specific rank. The form found in the Belgian Congo, Angola, French Equatorial Africa and West Africa he evidently considers as the true *palpalis*. As far as I can see, *martinii* can only be distinguished from *fuscipes* by the shape of the outer shoulder of the paramere : it is rounded in the former and more pointed in the latter. Although I have not had an opportunity of examining the ♂ terminalia of specimens of *palpalis* from Kasanga itself, I have examined the terminalia of specimens of *palpalis* from Kipili, 60 miles north of Kasanga, and those of specimens from Abercorn, south of Kasanga, which Mr. Potts very kindly sent me. The study of the paramere of these specimens, as well as that of those from the other localities noted, has convinced me that, as there is so much variation in the shape of the outer shoulder of the paramere, its neck-like extension and the expanded end, the structure of the paramere cannot be used to define species. I believe that the eastern form of *palpalis* is best regarded as a single variety *fuscipes* Newstead.

Glossina palpalis var. **wellmani** Austen (western form). DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Fig. 9, *h*, *i*. Although the anal cerci vary much in length, especially that of the tooth-like process, there is in this form no shelf on the inner side near the end. *Phallosome.* Figs. 10, *a* ; 15, *a*. The two posterior rod-like plates on the phallosome are in this form poorly developed (fig. 15, *b*) ; otherwise the phallosome is exactly similar to that of the type and eastern form *fuscipes*. *Paramere.* Fig. 15, *d*. There is no external shoulder on the outer side of the paramere, the neck-like extension is short, and the expanded end plate is wide and usually has 3–4 hairs on it. ♀. Fig. 15, *d*. The ♀ terminalia are very like those of the other two forms ; there are, as a rule, no hairs on the membrane between the two parts of tergum nine.

I have studied the terminalia of many specimens of *palpalis* from most parts of West Africa, and find that they all have the characters noted above. The question naturally arises as to what varietal name should be given to this form. I have been fortunate in being able to study the ♂ terminalia of a paratype specimen of *G. palpalis* var. *wellmani* Austen, which, as it will be remembered, was recorded from the Katumbela River, Angola, and I find that the ♂ terminalia are identical with those of specimens from the Gambia, Sierra Leone, etc. The markings on the mesonotum of this variety show a considerable reduction from that usually noted in *palpalis*. I believe, therefore, that it will be best to regard the western form of *palpalis* as the var. *wellmani*, and to note that it has two colour forms, a light and a dark, the latter, with the characteristic mesonotal markings, being the more typical north-western one. Professor Roubaud has kindly sent me a ♂ and ♀ from Grebon (Oubangui), French Equatorial Africa, and I note that the ♂ terminalia of this form are intermediate between those of the type form and the western form.

The conclusions reached as a result of the study of the three forms of *palpalis* may be summed up as follows :—*Glossina palpalis* R.-D. (type form).

This form is found in the northern, central and parts of the western Belgian Congo ; on the western side it is linked up with the West African form var. *wellmani*. *Glossina palpalis* var. *fuscipes* Newstead (eastern form). This distinct variety is widely distributed along the eastern boundary of the Belgian Congo,

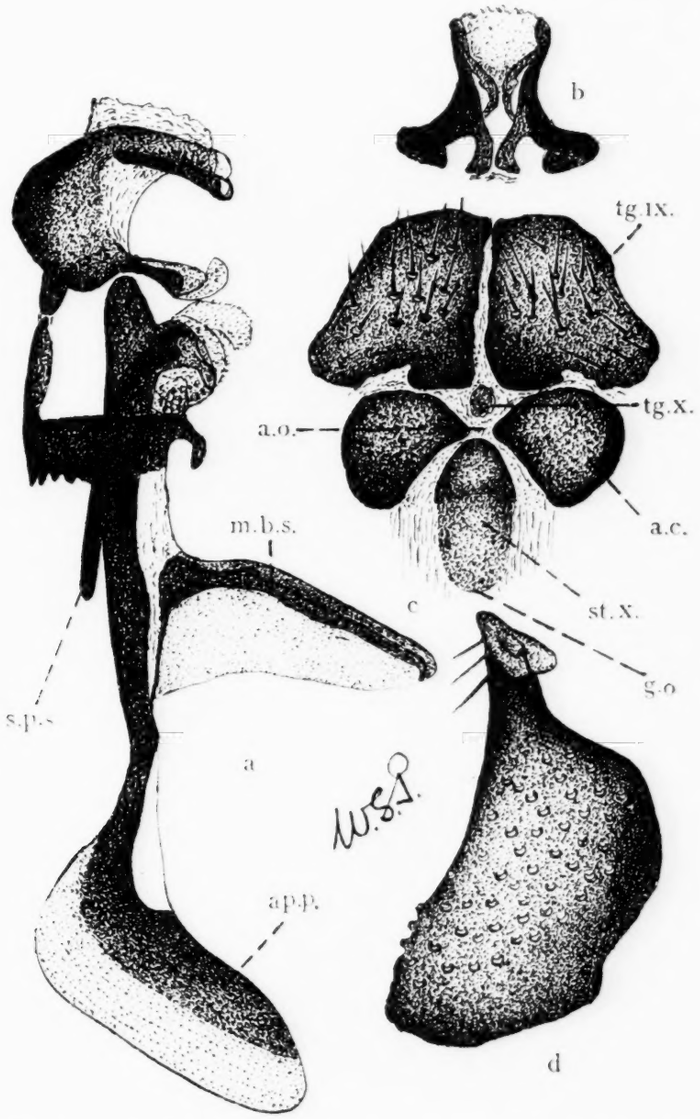


FIG. 15. *a*.—Phallosome of *palpalis* var. *wellmani*, the western form from a specimen from Baro, N. Nigeria ; *b*.—Enlarged ventral view of end of phallosome of same showing the small plates ; *c*.—Terminalia of ♀ ; in most of the specimens examined there were no small bristles between the two parts of tergum nine ; *d*.—Left paramere of same ; note the complete absence of a shoulder on the outer side.

from the Anglo-Egyptian Sudan in the north to Rhodesia in the south ; it is the common and only variety in the southern provinces of the Congo. This variety exhibits great variation in the structure of the paramere. *Glossina palpalis* var. ***wellmani*** Austen (western form). This distinct variety is found widely

distributed in West Africa, and extends south into Angola ; in French Equatorial Africa and the western Congo it is the predominant form.

DISTRIBUTION. HABITS. IMPORTANCE. The distribution of *palpalis* (including the two varieties) is very extensive. Its northern limit extends from the Senegal River in the west to the Bahr-el-Ghazal in the east ; in the south it extends from Angola across to the frontier of Northern Rhodesia, up along

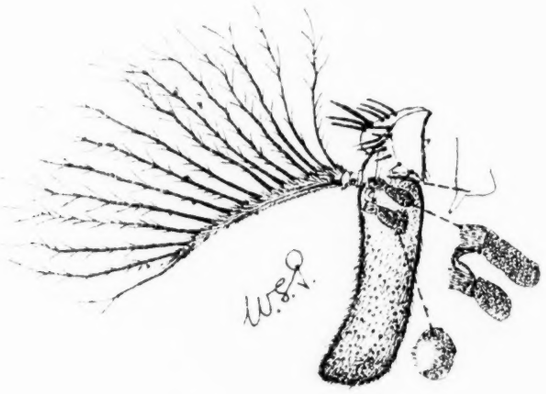


FIG. 16. Antenna of ♂ *tachinoides* ; peg and sensoria shown enlarged.

Lake Tanganyika and Lake Victoria to Lake Rudolph. It is not possible for me to summarize in a few sentences the voluminous literature dealing with the habits and life-processes of this important species. Briefly stated, *palpalis* is largely dependent on the presence of water and shade, and though it has been found some distance from water this is not its usual haunt. It is an important transmitter of pathogenic trypanosomes to men and animals.

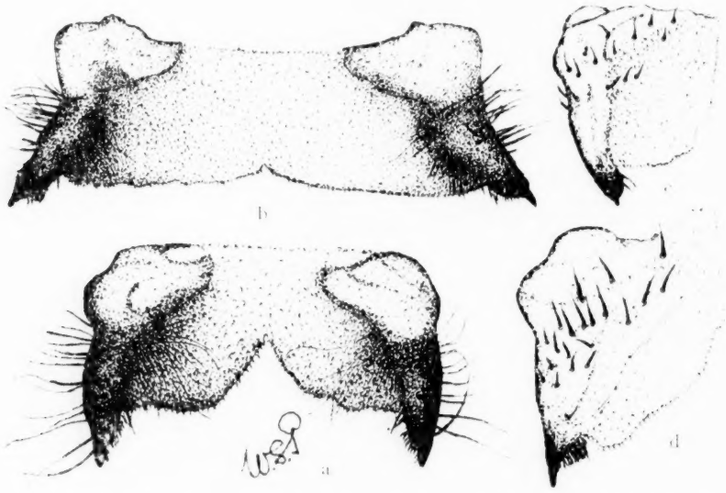


FIG. 17. *a.*—Ventral view of extended anal cerci of *pallicera* ; note the shelf with stiff hairs ; *b.*—Ventral view of extended anal cerci of *tachinoides* ; *c.*—Dorsal view of left cercus of same ; *d.*—Dorsal view of left cercus of *pallicera*.

Glossina tachinoides Westwood. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A small species, with distinct and sharply defined brown interrupted abdominal bands (unusual markings for species of this group). The hind tarsi are all dark. The third segment of the antenna (fig. 16) is rounded at the end and has no long hairs along its anterior border; the aristal hairs and their branches are relatively long. DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Fig. 17, *a*, *c*. The anal cerci

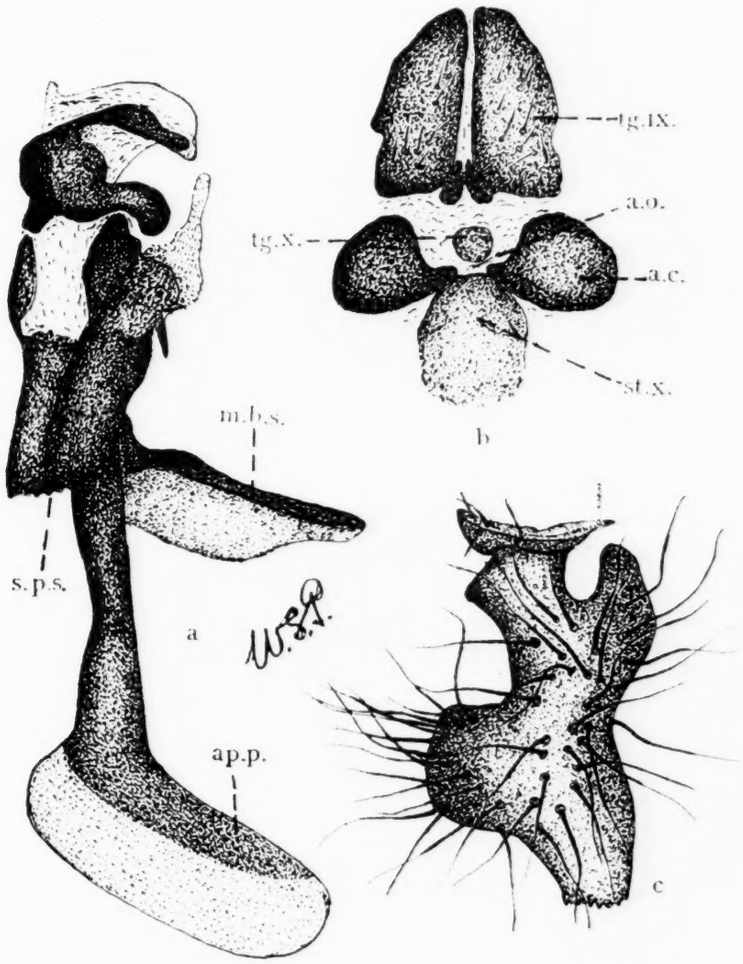


FIG. 18. *a*.—Phallosome of *tachinoides*; lettering as in fig. 3, *a*; *b*.—Terminalia of ♀; lettering as before; *c*.—Left paramere; note the long shoulder on the outer side and the pointed projection on the inner side near the expanded end.

are small, especially the tooth-like external process. *Phallosome.* Fig. 18, *a*. The phallosome is very similar to that of the other species of this group. *Paramere.* Fig. 18, *c*. The paramere, when dissected off and mounted without compression, has a very characteristic shape. The outer shoulder is long and rounded, and the neck short and wide, with a pointed projection on the inner side; the expanded end is long, wide and saucer-shaped, with 3–4 hairs. ♀. Fig. 18, *b*. The two parts of tergum nine are long and rather narrow.

DISTRIBUTION. HABITS. IMPORTANCE. *G. tachinoides* is widely distributed in West Africa, extending from Senegal in the west to the shores of Lake Chad in the east ; it is abundant in Northern Nigeria, and has been recorded from the French Congo. Carter recorded it from the Haushabi country and other localities in south-western Arabia, especially on the banks of the Wadi Tiban ; the writer was at Dthala (90 miles inland from Aden) and on the lower reaches of the Wadi Tiban, and did not see or hear of any biting fly which might have been a species of *Glossina* ; it has not been recorded from this locality since. Like *palpalis*, it is mainly restricted to the neighbourhood of water with dense vegetation, but it is also found in more open country with sparser vegetation ;

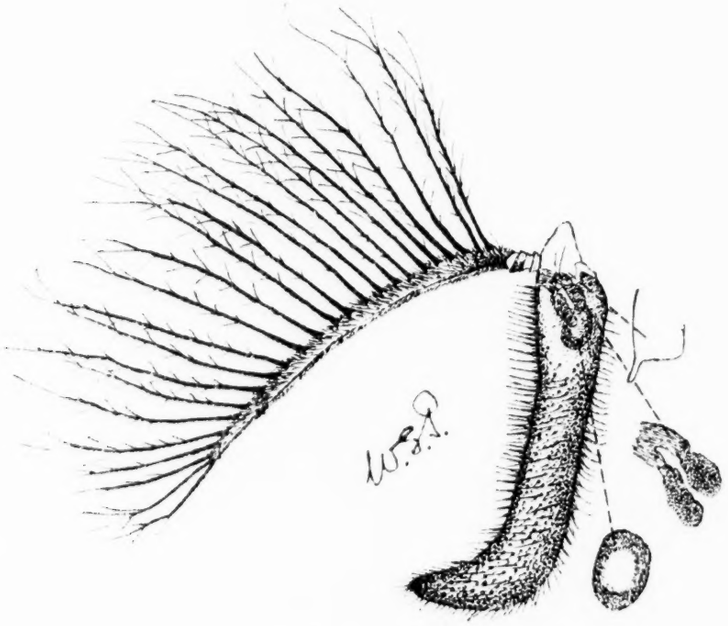


FIG. 19. Antenna of ♂ *pallicera* ; peg and sensoria shown enlarged ; note the long, narrow, markedly pointed and projecting end of third segment, and the very long hairs along the anterior border.

Roubaud points out that it has greater powers of resisting high temperature than *palpalis*. It is an important transmitter of pathogenic trypanosomes to man and animals.

Glossina pallicera Bigot. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A medium-sized species, the dorsal surface of the abdomen dull brown ; in the ♂ most of the terga are pale in the middle, whereas in the ♀ only the proximal ones are pale. Third segment of antenna (fig. 19) long and narrow, the end pointed and bent forward, with long hairs on the anterior border. DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci*. Fig. 17, *a*, *d*. Relatively short, broad plates, ending rather abruptly in a stout, pointed, inwardly bent tooth-like process ; on its inner side there is a

wide shelf armed with short stiff brush-like hairs. *Phallosome*. Fig. 20, *a*. Very similar to that of *palpalis* var. *wellmani*, the part carrying the ejaculatory duct relatively long. *Paramere*. Fig. 20, *c*. Similar to that of *palpalis* var. *wellmani* ; the expanded end is divided, and there is usually only one hair on

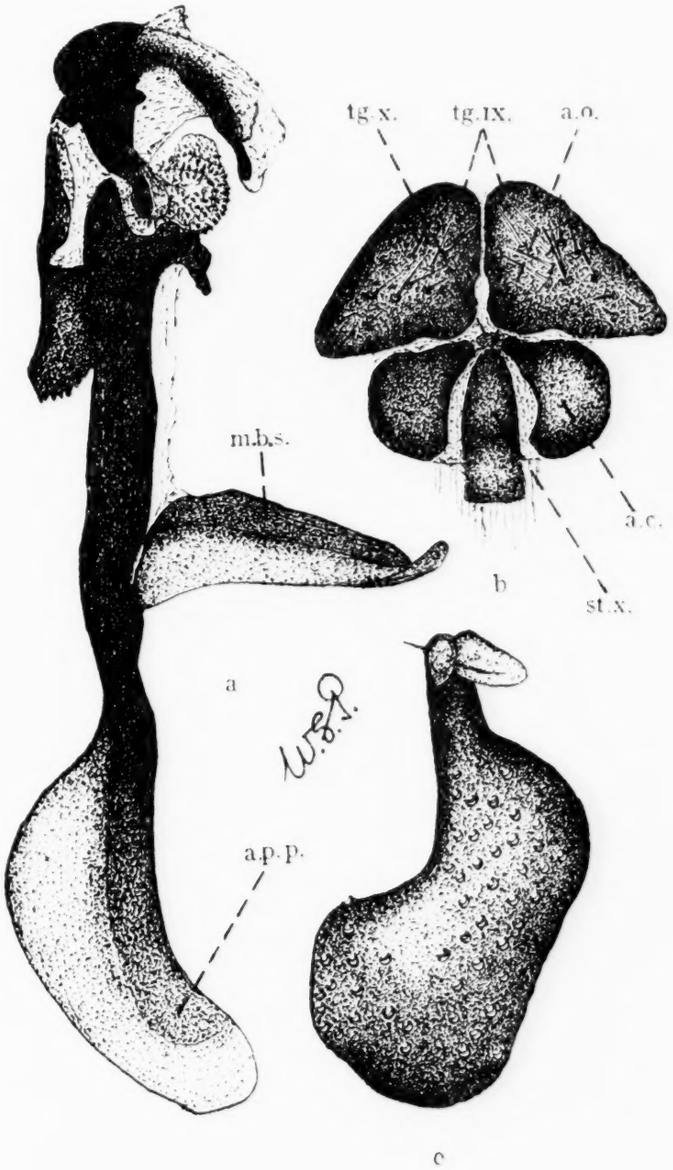


FIG. 20. *a*.—Phallosome of *pallicera* dissected out of the ninth tergo-sternum ; lettering as in fig. 3, *a* ; *b*.—Terminalia of ♀ ; lettering as before ; *c*.—Left paramere.

the inner side. ♀. Fig. 20, *b*. The two parts of tergum nine are triangular-shaped.

DISTRIBUTION. HABITS. IMPORTANCE. *G. pallicera* is a western species and has been recorded from Sierra Leone (Firo), Liberia, Ivory and Gold

Coasts, Southern Nigeria, Cameroons, French Equatorial Africa and the northern Belgian Congo (River Sabkuru). It is a relatively scarce species, except in certain localized areas. It is found on the banks of rivers and streams in dense vegetation, often along with *palpalis*. As far as is known, it is not of any economic importance.

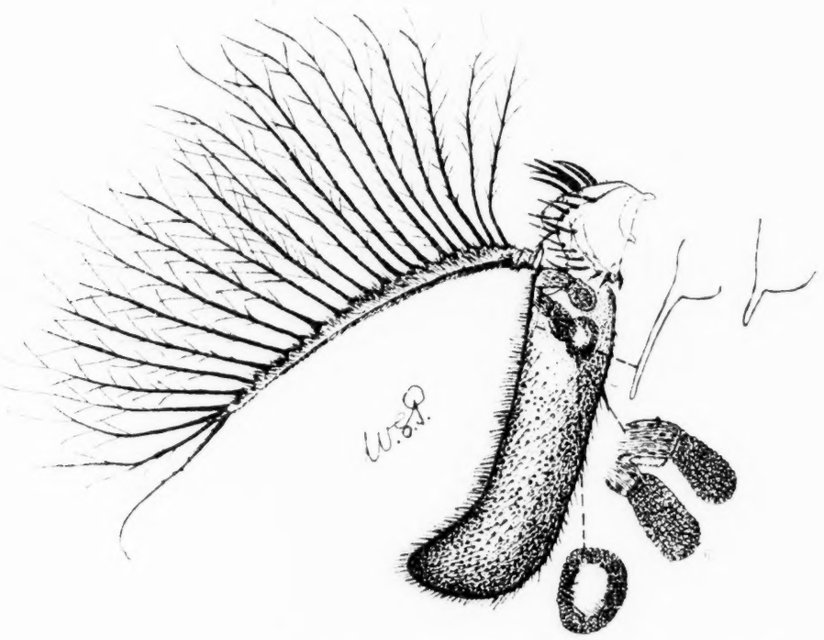


FIG. 21. Antenna of ♂ *caliginea*; peg and sensoria shown enlarged.

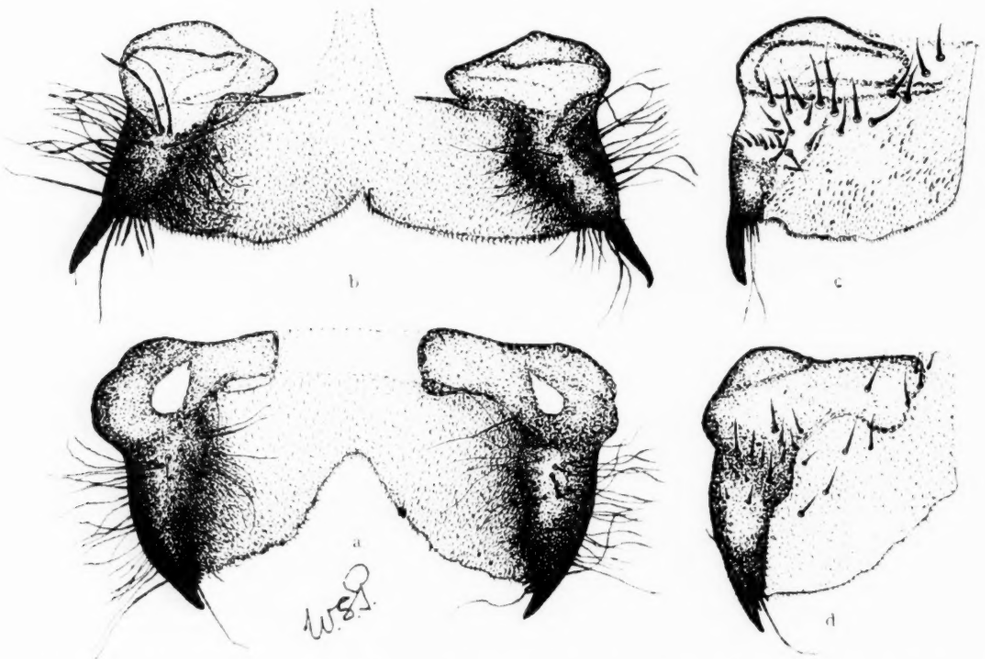


FIG. 22. *a.*—Ventral view of anal cerci of *newsteadi*; *b.*—Ventral view of extended anal cerci of *caliginea*; note the long claw-like process; *c.*—Dorsal view of left cercus of *caliginea*; *d.*—Dorsal view of left cercus of *newsteadi*.

Glossina caliginea Austen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A medium-sized to rather large species, distinctly larger than most specimens of *palpalis*. Third segment of antenna (fig. 21) wide, the blunt end slightly turned forward, without long hairs along

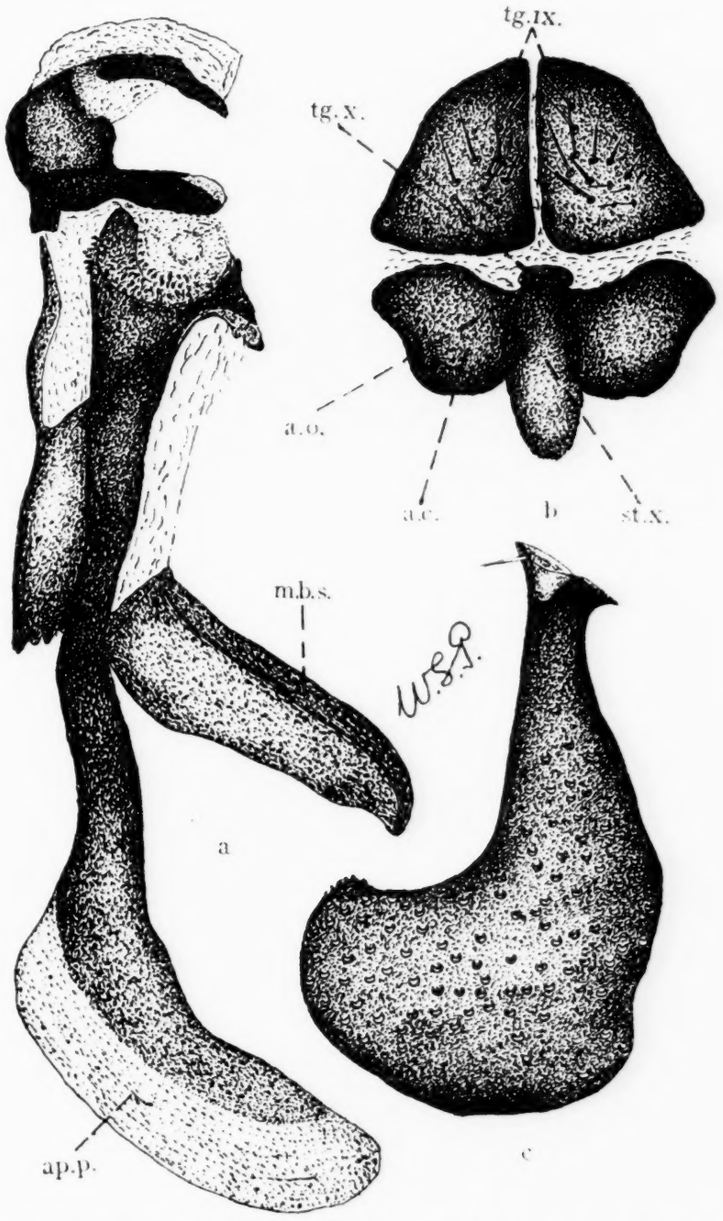


FIG. 23. *a*.—Phallosome of *caliginea* dissected out of ninth tergo-sternum ; lettering as before ; *b*.—Terminalia of ♀ ; lettering as before ; *c*.—Left paramere.

anterior border ; the aristal hairs long, with long branches ; peg of second segment of antenna with a long process (varying in length, fig. 21). Abdomen brown, the proximal part pale, as in *pallicera*. Its larger size and different antenna readily help to distinguish it from *pallicera*.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Fig. 22, *b*, *c*. Rather short and wide, ending on the outer side in a long, slightly bent process. *Phallosome.* Fig. 23, *a*. Very similar to that of *palpalis* var. *wellmani*; the finger-like membraneous process is long, but it should be noted that it is not peculiar to this species. *Paramere.* Fig. 23, *c*. The shoulder is wanting, the neck is long, and the expanded end is small, with a single hair on inner side.

DISTRIBUTION. HABITS. IMPORTANCE. *G. caliginea* is also a western species and has been recorded from the Gold Coast and many localities in Southern Nigeria and the Cameroons. It is found along rivers, and has been recorded as attacking people in boats. It is not known to be of any importance.

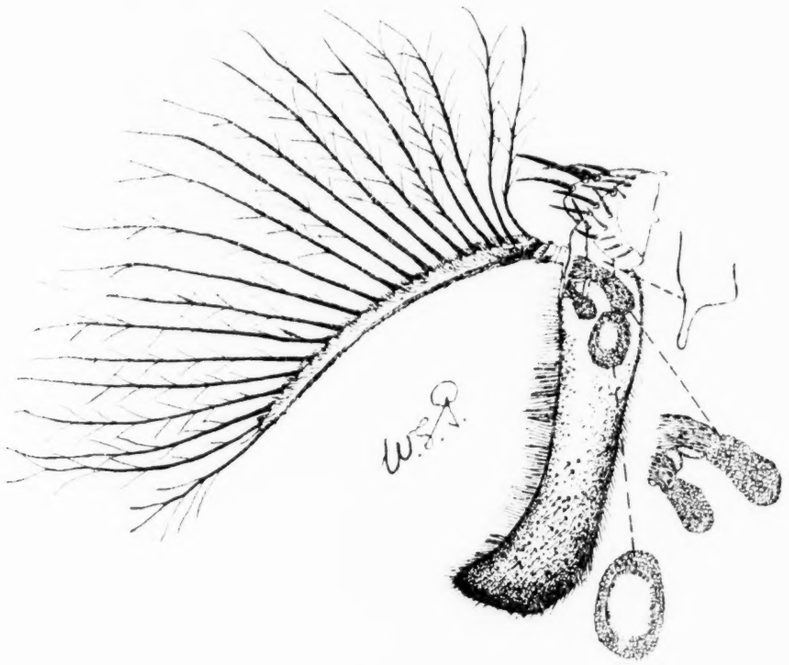


FIG. 24. Antenna of ♂ *newsteadi*; peg and sensoria shown enlarged.

Glossina newsteadi Austen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂, ♀. A medium-sized species, resembling *pallicera* and *caliginea*; the abdomen is brown and terga 1 and 2 have a light oval spot. Third segment of antenna (fig. 24) long and rather narrow, the end pointed and turned forward; the anterior border has long hairs. DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. *Anal Cerci.* Fig. 22, *a*, *d*. Rather short and broad, ending externally in a stout blunt process. *Phallosome.* Fig. 25, *a*. Very similar to that of *palpalis* var. *wellmani*. *Paramere.* Fig. 25, *c*. Shoulder wanting, neck long, ending in a snout-like expansion. ♀. Fig. 25, *b*. The two parts of tergum nine are short and wide.

DISTRIBUTION. HABITS. IMPORTANCE. This species was discovered by Dr. Schwetz in the lower course of the River Lomami, and especially on the banks of the small tributary Elipa, where it is abundant. A few other specimens were

also found in the Lower-Aruwimi (Bas-Aruwimi) below Banalia, and in Monga (Uele). In the Ituri forest it was taken near the village Bafwayabo below Bomili, and between the Rivers Okelu and Tshopo. Its haunts appear to be those of *palpalis*. It is believed to be of no economic importance.

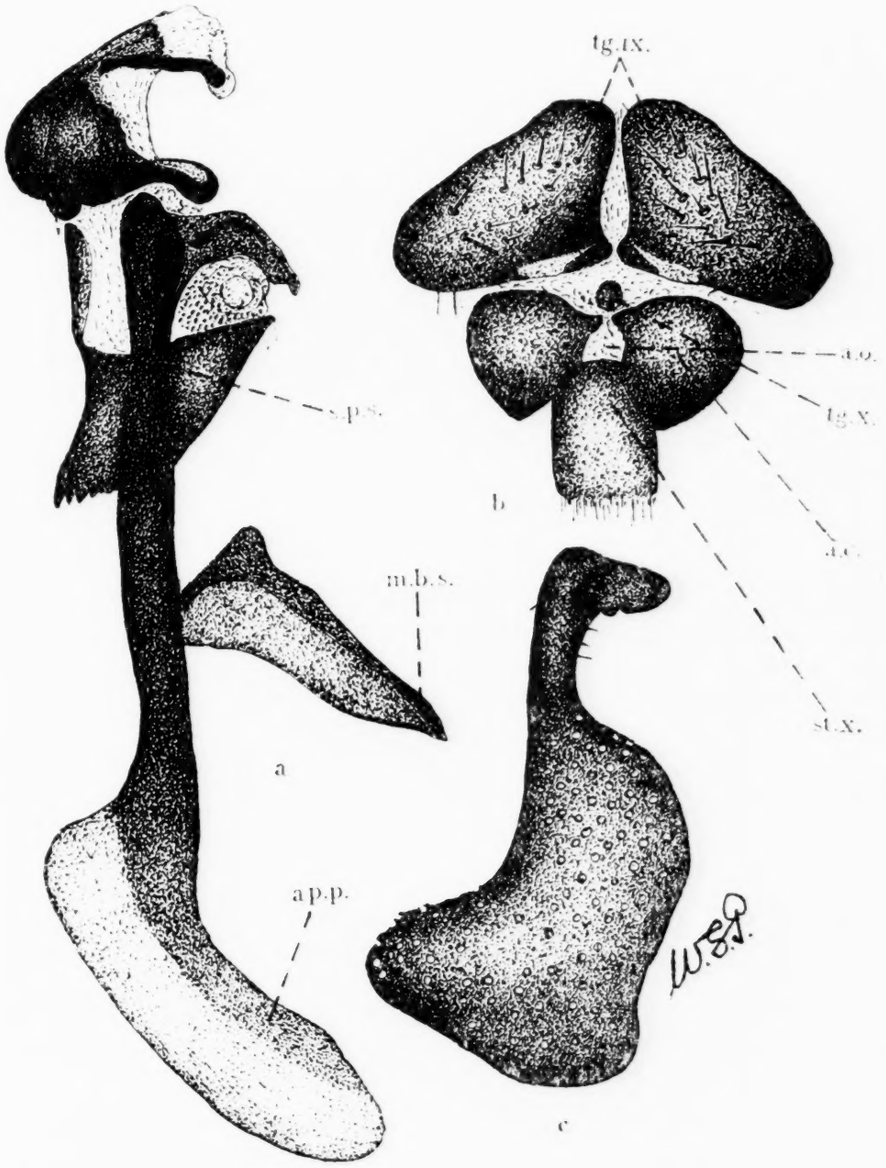


FIG. 25. *a*.—Phallosome of *newsteadi* dissected out of ninth tergo-sternum; lettering as before; *b*.—Terminalia of ♀; lettering as before; *c*.—Left paramere.

This concludes my revision of the species of *Glossina*. When I have completed my studies of the terminalia of the parasitic Muscidae, I propose to refer briefly to the systematic position of the genus *Glossina* and to the relationships of the species to each other.

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(Concluded)

FRAMBOESIA TROPICA—A SHORT REVIEW OF A COLONIAL REPORT CONCERNING STATISTICS AND *HIPPELATES FLAVIPES*

BY

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(*Received for publication* 13 *July*, 1936)

During the last century segregation was made compulsory in the West Indies for patients having the granulomatous eruption of yaws (framboesia tropica), and numerous asylums were built for their accommodation.

In the year 1911, I made a statistical study of all the patients who had been isolated during the years 1882-1910 in the yaws asylum in the small island of St. Lucia. This was published by the local government in my report 'for the half-year ending September 30th, 1911.' Eight detailed Tables of statistics appear in the report and show the incidence for sex and age, the urban or rural distribution of the homes of the patients, the monthly rates of admission, and the relation of these to rainfall.

Yaws, at one time very prevalent, is now, owing to the discovery of effective treatment, a disappearing disease, and the asylums of the West Indies have been closed because of the great decrease in the number of patients. Statistics, therefore, will not be compiled in the future, and it is improbable that many records of the past have been preserved.

It will, therefore, be of interest to give a summary of the statistics which lie buried in this old report, and a few of the conclusions which were drawn from them.

St. Lucia is one of the Windward Islands, its greatest length being about 27 miles, and its maximum width 14 miles. It is estimated to have a superficial area of 238 square miles. The only town is Castries, the port of the island, and during the years with which the investigation was concerned about one-sixth of the population of the island was living in this town in an area of 0·16 square miles. There were eight villages of varying size situated around the coast; and in the interior the population was scattered among the cocoa and sugar-cane estates or on small agricultural holdings. There were dispensaries in all districts in charge of Government medical officers, and the police and wardens of the villages and rural areas kept a look-out for cases of yaws and were under orders to send all persons suspected of having the disease to the medical officers for notification and segregation.

The registers of the yaws asylum were kept in the usual manner, the name, sex, age, date of admission, address and date of discharge of each patient being recorded.

Incidence for sex. There were 2,353 patients admitted to the asylum during the years 1882–1910, and of these 1,444 were males and 909 were females. The average population in the island during this time was 20,839 males and 22,519 females, whose ages were 60 or under. These figures give a percentage liability to yaws of 66 for males and 34 for females. There is a small error in this, because for the first decade of life there were slightly more males than females, and many cases of yaws occur during this decade.

Incidence for age. The following Table gives the percentage of the total patients for each decade of life.

Age	0–10	11–20	21–30	31–40	41–50	51–60
Percentage of patients ...	43·6	26·1	12·9	11·5	4·5	1·1
„ „ liability ...	27·6	21·6	14·2	20·4	11·4	4·4

As the population in the lower decades of life is far greater than in the higher, the percentage of the patients does not necessarily represent the ' percentage of liability ' to the disease.

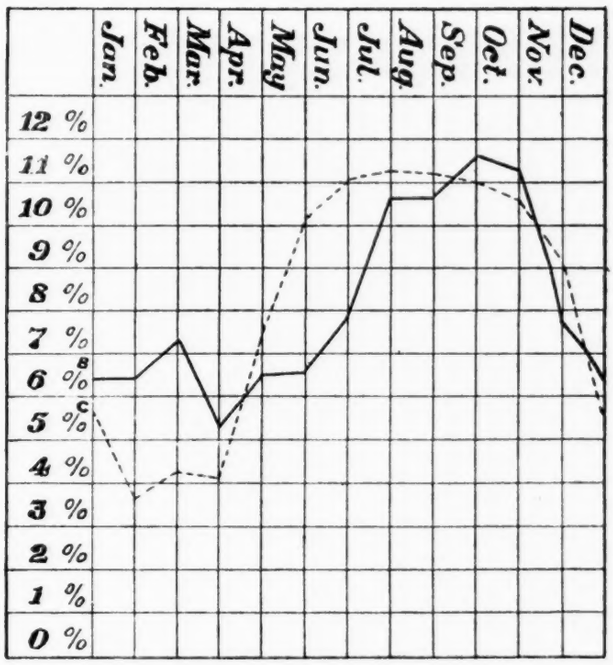
This was determined relative to the numbers of the population for each decade and is given above.

Urban and rural incidence. The patients were divided into three categories according to the areas from which they were sent : from the town of Castries, from village dispensaries, and from agricultural estates and small holdings. The following is the percentages of admissions for these areas :—

Town of Castries	3·4 per cent.
Village dispensaries..	33·2 „
Agricultural estates, etc.	63·2 „

It was difficult to correlate these figures with the numbers in the population in each area ; but a Table is given which shows that the average number of cases per 1,000 of the population was 0·36 yearly for the town of Castries and ranges from 0·8 to 7·4 for various villages and agricultural areas. It is almost needless to state that some of the patients may have acquired the disease away from their homes.

Relation to rainfall. Two Tables are given : one recording the percentage of the total patients admitted during each month of the year, and the second giving the average monthly percentage of the total rainfall. The graph below compares these. It will be seen that the number of admissions begins to increase six weeks to two months after the onset of the rainy season.



------ *Monthly Percentage of Yearly Rainfall*

—— " " " *Total Admissions*

Hippelates flavipes. The report under review gives a description of this small oscinid fly and its habits ; therein it is called the ' ulcer fly.' Specimens were sent to the British Museum (Natural History) in 1911, and were identified as ' *Osinis pallipes*,' and in a paper by the present author (Nicholls, 1912) the fly masquerades under this name. Graham-Smith also received specimens about this date, and in 1930 pointed out the wrong identification of the fly and stated that the correct name is *Hippelates flavipes.* The St. Lucia report states : ' In the West Indies I believe the majority of cases of framboesia are caused by a certain little fly inoculating surface injuries. . . . There are very few to be seen in Castries and there only in the outskirts of the town. They are very numerous in country districts, and can be seen hovering around the bare legs and arms of the labourers, searching for abrasions and possibly the secretions of the sweat and sebaceous glands. In cases of yaws, leprosy, syphilis and other conditions, in which there is considerable discharge from extensive areas, they swarm around the patients especially following the rains when their breeding season occurs. . . .

' The persistence of this little fly is extraordinary. As soon as an abrasion occurs or an ulcer is exposed numbers of them immediately alight upon it, and they must be brushed off by actually touching them, and they will immediately return ; they even try to insinuate themselves under dressings. They engorge themselves with pus, blood or serum until their abdomens are greatly distended. The distance they will fly in search of food may not be great, but they will follow a man or animal, which affords them an abundance of food, a long way.

' The yaws asylum is an excellent place to study the ulcer fly ; every exposed and moist ulcer or sore has many flies upon it or hovering around it. The eruptions of yaws tend to become very hard and dry ; the flies search around them, and if a scab is by chance or purposely removed or even slightly raised so that a little serum, blood or a moist surface is exposed, they will feed upon it, forcing their probosces under the scab. They continually pass from patient to patient, and any visitor to the asylum is carefully searched for skin abrasions by these little pests. I have frequently isolated pyogenic organisms from these flies.'

It is suggested in the report that the spread of yaws in St. Lucia depends principally upon nakedness, which renders a person liable to small injuries and abrasions, and the presence of the ' ulcer fly.'

The statistics reproduced were used to support this proposition. The higher incidence of the disease in males was attributed to the fact that nakedness and liability to injury are greater among the men working in the fields than among the women working in their homes and gardens, that boys are less clothed than girls, and, being more vigorous and adventurous in their lives, are more liable to small injuries. These three factors, nakedness, liability to injury, and the presence of *H. flavipes*, are far less frequent in urban than rural areas ; a labourer who works in the fields clad only in a loin cloth will put on a shirt and trousers when he visits a town, and there are no town occupations which render a person so likely to receive small injuries as a worker cleaning land of thorn scrub or working among the trash of cane-fields.

The curve of increasing admissions to the asylum following the rainfall curve (cf. graph) by an interval of about two months may indicate the time required for the breeding of swarms of *H. flavipes* and the incubation period of yaws. But very apparent signs of the disease are delayed in many patients, and when the patients are ineffectively treated the disease tends to become chronic ; consequently in whatever season the disease may be acquired there will be admissions or readmissions to the asylum at all seasons of the year. That the incidence of infection is even greater during the rainy season than was indicated in the graph is a reasonable deduction.

It does not appear necessary, for the transmission of this disease by *H. flavipes*, for the spironema to survive long on the body of the fly, and still less to have a life-phase within it, because the flies pass rapidly from one person to another in their search for food.

Length of time patients remained in the asylum. There are two Tables in the report under review relative to this. The first shows that 48·06 per cent. of the patients remained in the asylum for less than 100 days, and that 51·94 per cent. remained for periods ranging from 100 days to over 400 days. The second Table shows the average number of days that patients of three age-groups remained in the asylum. The following summarizes it :—

Age	1–10	11–20	Over 20
Average no. of days...	192	173	143

These Tables are of interest now that the disease can effectively be treated.

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THE BRITISH SPECIES OF THE SUBFAMILY SARCOPHAGINAE, WITH ILLUSTRATIONS OF THE MALE AND FEMALE TERMINALIA

(*Continued from page* 201)

BY

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AND

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(*Received for publication 22 July*, 1936)

Sarcophaga sinuata Meigen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂. This is another of the species with a single slight distinctive character which enables it to be identified at once. On the front of the middle femora the apical third is entirely free of setae, and is covered with fine decumbent golden or brownish hairs, in both sexes. We know of no other European species with anything similar.

It is a medium-sized species, 7 to 8 mm. long ; frons at narrowest about two-thirds of eye-width ; lateral verticals strong and long ; antennae with third joint about one-third longer than second ; genae about one-third eye-width ; few genal setae, of which only about three are long and strong. General body colour black with silvery tomentum, three broad black stripes on thorax and usual abdominal tesselation ; no presutural acrs. ; three postsutural D.C. (Böttcher says sometimes four) ; a pair of strong reflexed and crossed scutellar apicals, one pair of long strong preapicals, one pair of strong basals, and a pair of finer discals. There are strong hind marginals on second visible abdominal tergum ; both genital segments shining black and free from tomentum, with some long pilosity and a row of fine marginal bristly hairs on first ; sternites with long upstanding pilosity ; apical sternum with an indefinite ' brush ' combined with a marginal fringe of longer irregular setae. Wings with costal thorn ; third and fifth costal sections about equal ; first longitudinal vein bare, third with usual few setae on basal portion. LEG CHAETOTAXY. Fore femora with usual postero-ventral series strongly developed, and some long pilosity behind ; mid femora with antero-ventral series incomplete, only one long bristle on basal half and a row of variable longish ones from middle to tip ; postero-ventral series complete, with medium-length setae throughout and no suggestion of a comb ; hind femora with a row of about six long strong setae on antero-ventral aspect reaching neither end, and with a full postero-ventral series of rather long fine bristles ; hind tibiae with fringe of long hairs on postero-ventral aspect, and a few such hairs antero-ventral.

♀. As stated above, the ♀ is as readily recognized as the ♂ by the same character of the golden patch on the mid femora, and it is very like the ♂. The frons is wider than an eye—perhaps one-quarter wider ; the apical scutellars fail ; the sixth tergum is widely divided above, with a bare rather shining space between the two halves ; and the hind margin of each half has a row of bristles. LEG CHAETOTAXY. Much the same as in the ♂, only sparser, and on the middle femora the apical portion of both series consists of shorter setae forming ill-defined combs ; postero-ventral series of the hind femora reduced to three or four long bristles, and there is no pilose fringe on the tibiae.

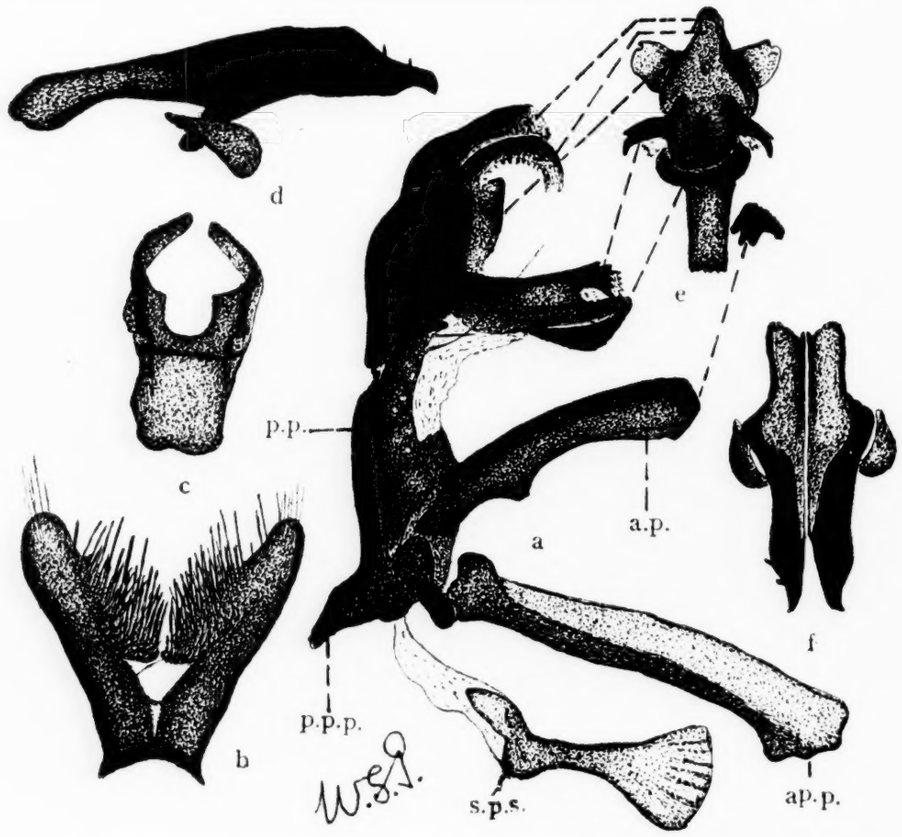


FIG. 1. *a.*—Phallosome and one paramere of *sinuata* in side view ; *a.p.*—Anterior part of paramere ; *ap.p.*—Apodeme of phallosome ; *p.p.*—Posterior part of paramere ; *p.p.p.*—Posterior process of phallosome ; *s.p.s.*—Sperm pump sclerite ; *b.*—Fifth sternum ; *c.*—Ninth tergo-sternum ; *d.*—Lateral view of anal cercus and distal segment of ninth coxite ; *e.*—Dorsal view of end of phallosome ; *f.*—Ventral view of anal cerci and distal segments of ninth coxites.

NOTES. *Sinuata* is not a common species, our series coming chiefly from by the River Lugg at Shobdon in Herefordshire, where it was not uncommon in August, 1934 ; we also have it from Upton Broad, Norfolk ; Chalford, Gloucestershire ; Water Eaton, near Oxford (J. Collins) ; and we have seen it from the New Forest (M. A. Sharp) ; Cambridge (F. Jenkinson) ; Bossiney, Cornwall (Rev. A. Thornley) ; Farnborough (J. W. Saunt) ; and Pulford, Cheshire (H. Britten). Dr. C. D. Day reports that he has taken it in Dorset. It is probably generally distributed but uncommon. Abroad it appears to be nowhere common,

but Böttcher records it from Germany, Austria, Hungary, Switzerland, France and Italy, and Lundbeck adds Denmark, Scandinavia up to middle, and Finland. Aldrich records it from U.S.A., where it seems to be widely distributed, and adds that it has been bred from *Melanoplus differentialis* ; but again we suggest that all records of parasitism in this genus should be treated with doubt, in view of the fact that the specimens are likely to have been present merely as scavengers, and the supposed host to have been dead when attacked.

Diagnostic Characters of Terminalia. ♂. Fig. 1. Each anal cercus is a long plate, closely united to its fellow for about three-quarters of its length. In profile (fig. 1, *d*) the cercus is seen to be a wide plate terminating in a short narrow free end which is bluntly pointed and turned upwards ; it bears on its

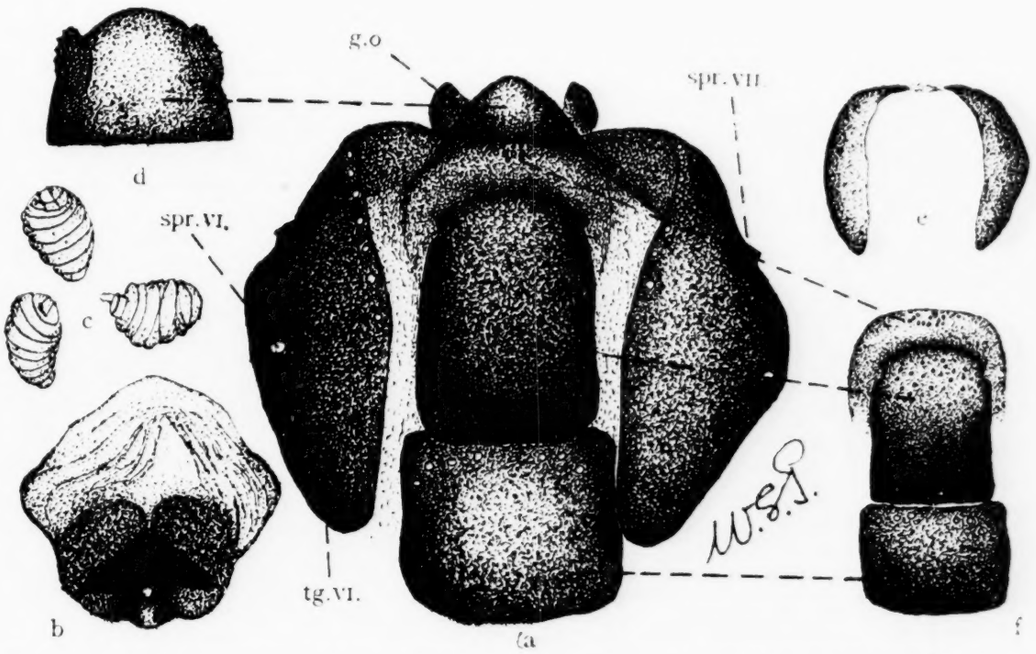


Fig. 2. *a*.—Ventral view of terminalia of ♀ *sinuata* to show diagnostic characters ; *g.o.*—Genital opening ; *spr.vi, spr.vii.*—Sixth, seventh spiracle ; *tg.vi.*—Sixth tergum ; *b.*—Signum ; *c.*—Spermathecae ; *d.*—Tenth sternum and anal cerci ; *e.*—Sixth tergum, to show that it consists of two distinct plates ; *f.*—Sixth, seventh, ninth (?) sterna.

ventral surface near the free end a few short stiff hairs. The ventral view of the two anal cerci are illustrated in fig. 1, *f*. The phallosome (fig. 1, *a*) is relatively short, ending in a short median process with a pair of wide side flaps (fig. 1, *e*). Projecting from the middle of the dorsal side there is a pair of long processes. The posterior part of the paramere (fig. 1, *a*) is a short plate with a bluntly pointed end, close to which are attached two long bent hairs ; the anterior part of the paramere is a long broad plate. Sternum 5 (fig. 1, *b*) has a short stem and long arms with numerous stiff hairs on their inner sides.

♀. Fig. 2. Tergum 6 consists of two separate plates, as already noted above ; tergum 7 is wanting ; sternum 6 (fig. 2, *f*) is a square plate ; sternum 7

(fig. 2, *f*) is much longer, and its distal end is surrounded by a lightly chitinized plate which may represent sternum 9. Sternum 10 (fig. 2, *d*) is a wide triangular plate with the small anal cerci attached to it. The signum is illustrated in fig. 2, *b*, and the spermathecae in fig. 2, *c*.

Sarcophaga albiceps Meigen. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂. *Albiceps* belongs to a group of species closely resembling one another and difficult to distinguish on external characters alone. They all agree in possessing 4 or 5 postsutural D.C., with only the two hinder ones long and strong. There are no marginal macrochaetae on the second visible abdominal segment ; no marginal setae on the first genital segment (seventh tergum), which is dusted with grey tomentum ; no setae on first longitudinal vein, and no presutural acrs. The terminalia are usually black in typical specimens, but more-or-less red forms do occur. In the key in ' British Tachinidae ' (1928), the group is represented by *teretirostris* Pand. alone, and *albiceps* would run down to it. It is nearly allied to *carnaria* L. and *vicina* Villen., which are distinguished amongst other characters by the possession of marginals on the second abdominal segment ; to *aratrix* Pand., which is readily recognized by the peculiar formation of the fifth sternum ; and to *scoparia* Pand., whose dense shaggy pilosity, especially that on the sternites, is distinctive. Since the key was published by one of the writers (C. J. W., 1928), however, the present species, *tuberosa* Pand. and *similis* Pand., have been found in this country, all belonging to the *teretirostris* group. They are all about the same size, being amongst the larger species and similar in appearance to *carnaria* L., and are easily identified if the terminalia be examined, though they are very much alike externally. The terminalia seem to show relationships to one another, as they are similarly complicated, and, as they cannot be described accurately, drawings are essential. We have attempted a key with which it may be possible to separate these four species, but most of the characters are rather variable and so are not wholly reliable.

1. Fifth sternum bearing a well-defined ' brush ' of short, thickly set setae, and also an irregular marginal fringe of longer thick bristles ; (second genital segment shining, and pilosity on this and at the base of the cerci not very long ; third tibiae with fringe of long hairs little developed ; none on the antero-ventral aspect, and the postero-ventral series scattered and not very long) ... ... *similis* (Meade ?) Pand.
   Fifth sternum without brush 2
2. Fifth sternum with only a slight fringe of short fine hairs ; second genital segment (tenth tergum) with some grey tomentum ; (pilosity on second genital segment and on base of cerci long and shaggy ; third tibiae with very long fringes, especially on postero-ventral aspect, where many hairs are half as long as the tibiae themselves and they are very numerous ; on the antero-ventral aspect they are also long and dense ; fourth sternum with very short sparse hairs) *albiceps* Mg.
   Fifth sternum with marginal fringe of short thick setae ; second genital segment shining 3

3. Hind tibiae usually with long fringes on both sides, though not so long as in *albiceps* ; fourth sternum with very short sparse hairs ; (second genital segment often red) ... ... *tuberosa* Pand.
Hind tibiae with fringes less developed, especially on antero-ventral aspect, where there are only a few short and irregular hairs ; fourth sternum with short but upright and distinct pubescence ; (second genital segment always black) ... *teretirostris* Pand.

Other characters of *albiceps* which are rather distinctive are the general reduction of the thoracic setae, the anterior postsutural D.C. and most of the presutural being sometimes not easily distinguished from the general clothing of hairs ; ocellars also reduced and quite inconspicuous ; antennae rather long, the third joint being nearly three times as long as wide. *Albiceps* and *tuberosa* are especially alike, but, apart from characters mentioned above, the general chaetotaxy in *tuberosa* is more developed, the antennae little more than twice as long as wide, and the leg fringes of long hairs all less developed.

Albiceps is one of the larger species, normally about 11 mm. long ; frons about three-fifths of eye-width ; no outer verticals ; genae with regular row of long fine setae, arista with long ciliation ; thoracic stripes strongly marked ; third costal section about equal to fifth and sixth combined ; no costal thorn ; only small apical scutellars. LEG CHAETOTAXY. Front femora with usual post-ventral series as long as usual, but sometimes fine and not clearly distinguished from the dense long general pilosity ; mid femora with a few irregular long setae on the antero-ventral basal two-thirds, and a fine comb at apex ; postero-ventral aspect with long dense fringes of hairs on basal portion, and a more strongly developed comb ; hind tibiae with irregular scattered antero-ventral row of long setae, otherwise with dense long hair fringes inside and out, and tibiae as above.

♀. Owing to lack of material in the allied species we are not yet able to make comparative descriptions of the ♀♀ of this group. We only know one ♂ of *similis* and no female ; we have some Continental specimens which we believe are *tuberosa*, but we are not quite certain ; and even of the better-known *teretirostris* we have insufficient ♀ specimens. We have, however, a ♀ *albiceps* taken in cop. from Italy, and some ♀♀ bred from larvae at Conoor, South India.

The ♀ is stout and rotund, almost cylindrical in section, and yellowish in tone, with all the markings very distinct. It agrees in most characters with the ♂, excepting for the usual sexual differences ; the antennae are long in both sexes ; the tendency to reduced chaetotaxy is more marked, especially in the Indian specimens ; fifth tergum has a row of strong marginals ; the sixth is joined above and consists of a single plate ; it is wide, and there is a row of long strong bristles along most of the hind margin but failing above ; the sterna are largely covered up, the sixth bears two or three apical bristles on each side, the seventh is more exposed and wide, with a row of about six small marginals. In the *tuberosa* ♀♀ mentioned, apart from the terminal plates showing some red colour, all the sterna are wider and more exposed, bearing

four hind marginal bristles on most, usually more on the sixth, and even as many as eight on the seventh. The frons of *albiceps* is narrow for a ♀, being less than eye-width ; the frontalia are less than twice the width of the orbits, and reddish, and the orbits bear golden tomentum. The *tuberosa* ♀♀ seem to have a rather wider frons, a little over eye-width, with frontalia wider in proportion and quite black. *Teretirostris* seems to have the frons of eye-width, and the frontalia black or perhaps dark red and double width of orbits ; sterna narrow as in *albiceps*. But all these characters are 'hair-splitting' and, we fear, rather unreliable ; without more material to study we cannot be sure that it is possible to distinguish the ♀♀ of this group on external characters.

Leg Chaetotaxy. Like the thoracal chaetotaxy, that of the legs tends to be reduced ; front femora with usual post-ventral series strongly developed, with no trace of long pilosity of the ♂ ; mid femora with both antero- and postero-ventral series consisting of three long strong bristles in basal portion, and a comb of very short rather fine setae in apical portion ; hind femora with a few irregular long strong bristles on antero-ventral aspect, and only two at base of postero-ventral aspect ; no long fringing pilosity anywhere on the legs, as the ♂ has. *Teretirostris* seems to have more development of the leg bristles throughout, and especially on the mid femora, where the postero-ventral series is complete, with about six long ones and a more strongly developed comb ; also there is some long pilosity behind the front femora and a little behind the hind femora at base ; perhaps the reduced chaetotaxy may enable ♀ *albiceps* to be recognized from *teretirostris*, although all chaetotaxical characters are especially variable and uncertain in this genus, in which, as it happens, we are particularly dependent upon them.

Notes. *Albiceps* occurs in Verrall's ' List of British Diptera ' and in earlier works on British flies ; but we had seen no British specimen which proved on dissection to belong to this species until one of us took a single ♂ on the South Downs at Clapham Wood on June 11th, 1935, which is still the only one known to us. It is an unusually widely distributed species, and according to Böttcher is especially common in many parts of Asia, and has been received from New Guinea and from various localities in North and East Africa ; Böttcher adds that it appears to occupy the same position in Asia as *carnaria* does in Europe, being the commonest species of *Sarcophaga* there. In Europe, too, it is widely distributed, but Lundbeck does not include it in his ' Diptera Danica,' and perhaps it is more a warm-country species. In the east there is a form with reddish terminalia and palpi, but the European examples appear to be always dark.

Diagnostic Characters of Terminalia. ♂. Fig. 3. The anal cerci are short and densely covered with long curly hairs (fig. 3, *c*) ; the free end of each is bent upwards in a short hook-like point ; the ventral view of the cerci is illustrated in fig. 3, *e*. The phallosome (fig. 3, *a*) is relatively short and ends in a long hood-like process. Projecting from the middle of the dorsal side (as

seen in side view) there is a characteristic long bilateral process. The curved serrated distal end of each process is seen in the dorsal view of the end of the phallosome in fig. 3, *f*. The posterior part of the paramere (fig. 3, *a*) is a short stout plate with a curved pointed end, near which there are two long hairs attached. The anterior paramere (fig. 3, *a*) is a longer plate with a bluntly rounded end. Sternum 5 (fig. 3, *b*) is a long Y-shaped plate, the two arms with numerous short stiff hairs along the inner borders.

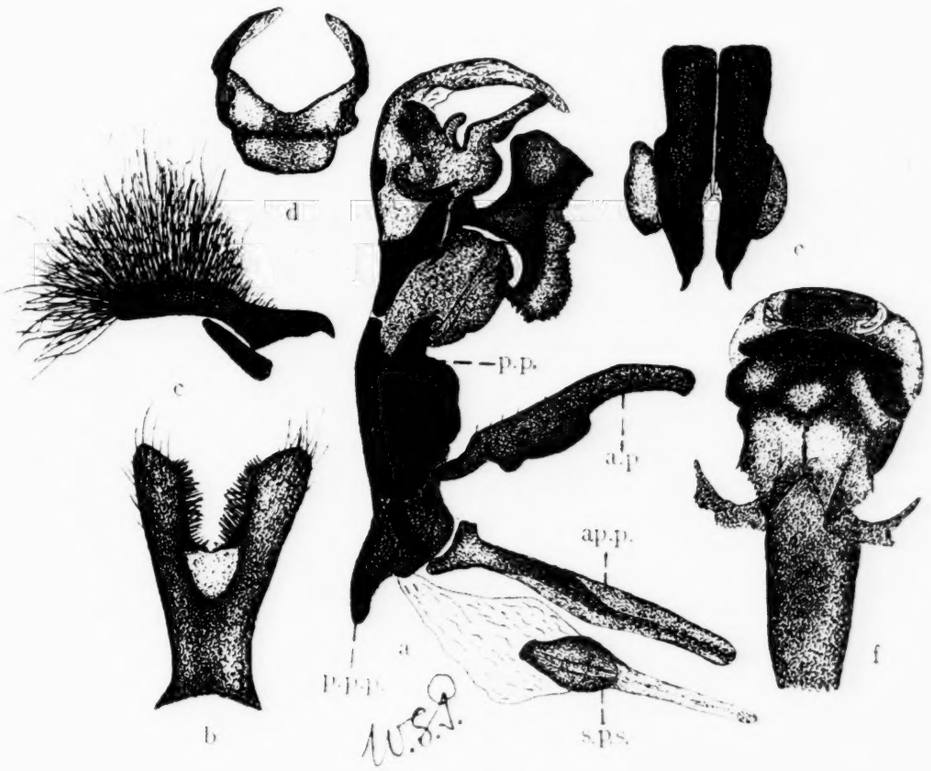


FIG. 3. *a*.—Phallosome and one paramere of *albiceps* in side view ; lettering as in fig. 1, *a* ; *b*.—Fifth sternum ; *c*.—Lateral view of anal cercus and distal segment of ninth coxite ; *d*.—Ninth tergo-sternum ; *e*.—Ventral view of anal cerci and distal segments of ninth coxites ; *f*.—Dorsal view of end of phallosome (from bred specimen, S. India).

♀. Fig. 4, *a*. Tergum 6 consists of a single plate ; tergum 7 of two widely separated long narrow plates. Sterna 6 and 7 are broad and short, especially the latter, and appear to be embedded in a broad, lightly chitinized plate, the distal end of which bears numerous fine hairs, and this part may represent sternum 9. Sternum 10 (fig. 4, *c*) is a wide, somewhat triangular plate, which has two smaller triangular plates attached to it, which form the posterior wall of the genital opening. The signum is wanting.

Sarcophaga setipennis Rondani. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂. This is one of a small group of species, possessing black terminalia and three postsutural D.C., which is readily

distinguished by the presence on the first longitudinal vein of a row of setae occupying most of its length. In this country there are only three species with this combination of characters, and *setipennis* is distinguished from the other two, *dissimilis* Mg. and *offuscata* Schr., by the greater width of the frons, which in this sex is just about equal to eye-width, whilst in the others it is barely half of the same. Further readily observed distinctions are that in *setipennis* there are longish setae on the lamellae of the fifth sternum forming a marginal fringe, whilst in the other two species there are closely set short setae forming the so-called ' brush ' ; also *setipennis* has no marginal bristles on the seventh tergum (first genital segment), and the others have distinct marginal series.

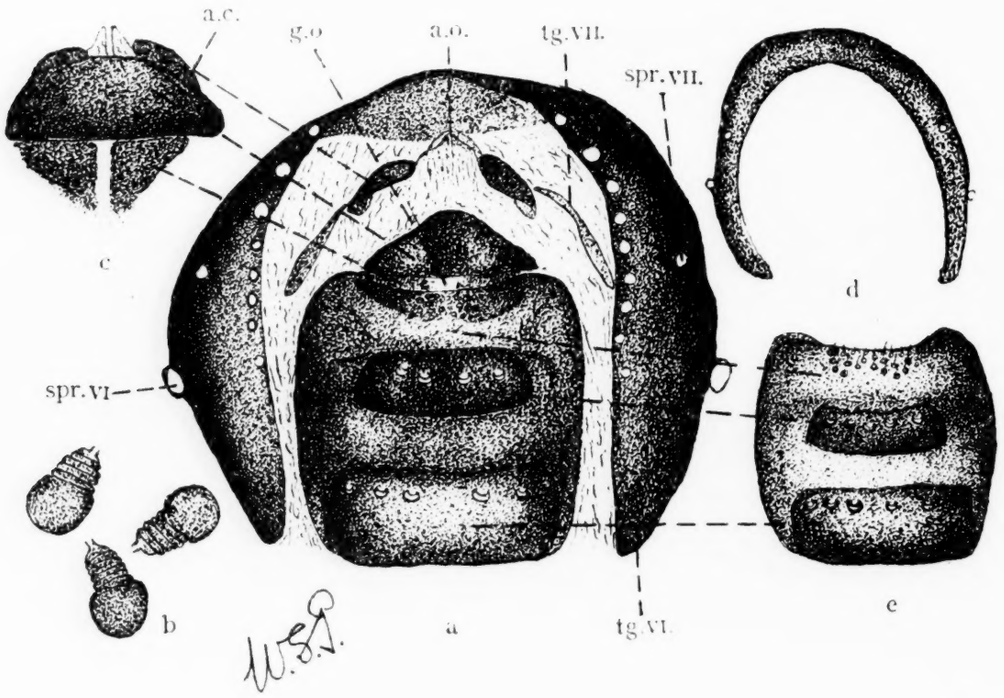


FIG. 4. *a*.—Ventral view of terminalia of ♀ *albiceps* to show diagnostic characters ; lettering as in fig. 2, *a* ; *b*.—Spermathecae ; *c*.—Tenth sternum and anal cerci ; note the divided plate attached to the former ; *d*.—Sixth tergum to show that it consists of a single plate ; *e*.—Sixth, seventh, ninth (?) sterna (from bred specimens, S. India).

Outer vertical bristle well developed ; antennae with third joint about half as long again as second ; arista with short ciliation ; genal bristles nearly in a row, about three large and strong ; thorax with presutural acrs. somewhat developed ; three distinct dark stripes ; scutellum with pair of long recurved apicals, strong preapicals and equally strong basals ; a pair of short discals only ; a pair of strong marginals on second visible abdominal tergum ; full series on margin of third and fourth ; none on first genital segment (seventh tergum) ; few scattered ones on second genital (tenth tergum), with some tomentum ; sterna with some longish pilosity ; strong costal thorn ; third and fifth costal sections nearly equal. LEG CHAETOTAXY. Front femora with usual full postero-ventral series ;

mid femora with a scattered antero-ventral row of shortish bristles and a more complete postero-ventral row ; no comb ; at the apical end of the postero-ventral series a row of close-set long thin bristles ; hind femora with a scattered, sparse, ventral series, and only three or four odd bristles on postero-ventral aspect.

♀. Very like the male except for usual sexual differences. It has the setose first long vein, and, as in the ♂, the frons is wider than that of the two associated species, being about a quarter more than eye-width, whilst in the others it is just about eye-width. The terminalia are not very conspicuous, but it is not difficult to note that tergum 6 is divided into two plates ; in the other two species it is a single plate. *Dissimilis* is a very shiny insect, the dark markings almost monopolizing the tergum with very reduced white tomentose markings at the sides ; *setipennis* has the more usual *Sarcophaga* pattern, with tomentum well developed. As usual in ♀♀ of this genus, the apical scutellars fail ; the sterna are nearly hidden ; there are strong bristles on the whole hind margin of tergum 6. Leg Chaetotaxy. Much the same as in the ♂ but reduced on the mid femora, where the postero-ventral series is sparser and shorter and forms no close-set row at apex.

Notes. *Setipennis* is a fairly common species and widely distributed, but we have not come across it anywhere in any great numbers, and at present it has not been found north of Norfolk. According to Böttcher it occurs widely in Germany and in Hungary, France, Italy and Spain ; Villeneuve has recorded it from Corsica, Pandellé from Poland, and it has been taken in Algeria. Lundbeck does not include it in his Danish species, so that it seems to be a more southern insect. It has been recorded by Giard as having been bred from a snail, *Helix acuta* ; but as usual one has to regard it as at least possible that the snail was merely carrion.

Diagnostic Characters of Terminalia. ♂. Fig. 5. Each anal cercus in side view (fig. 5, *e*) is a short broad plate sloping to the free end, which is bent upwards. The ventral view of the two anal cerci is illustrated in fig. 5, *d*. The phallosome is short, the distal end globular, with a pair of narrow serrated plates on each side of the middle line ; proximal to these and also on each side of the middle line there is a pair of wider processes ; in the dorsal view (fig. 5, *f*) it will be noted that the sides of the phallosome are serrated. Structurally the distal end of the phallosome of *setipennis* bears a resemblance to that of *clathrata* on the one hand and to that of *nigriventris* on the other ; the differences between them will, however, be readily noted by comparing fig. 5, *a*, with the illustrations in our two previous papers. The posterior part of the paramere of *setipennis* (fig. 5, *a*) is a long broad plate, with the end bluntly pointed and with two long curved hairs on the dorsal border ; the anterior part (fig. 5, *a*) is a long broad plate. Sternum 5 (fig. 5, *c*) is Y-forked, the long arms having many long stiff hairs along the inner borders and the stem being short and wide.

♂. Fig. 6, *a*. Tergum 6 consists of two distinct plates joined by membrane ; tergum 7 is wanting. Sternum 6 is a square plate, sternum 7 longer and narrower, and at the distal end of it there is a lightly chitinized plate, possibly sternum 9, with some more strongly developed chitin in the middle and a row of microscopic hairs at the end. Sternum 10 (fig. 6, *c*) is a triangular plate, with a small plate attached to and forming the posterior wall of the genital opening. The signum is illustrated in fig. 6, *b*.

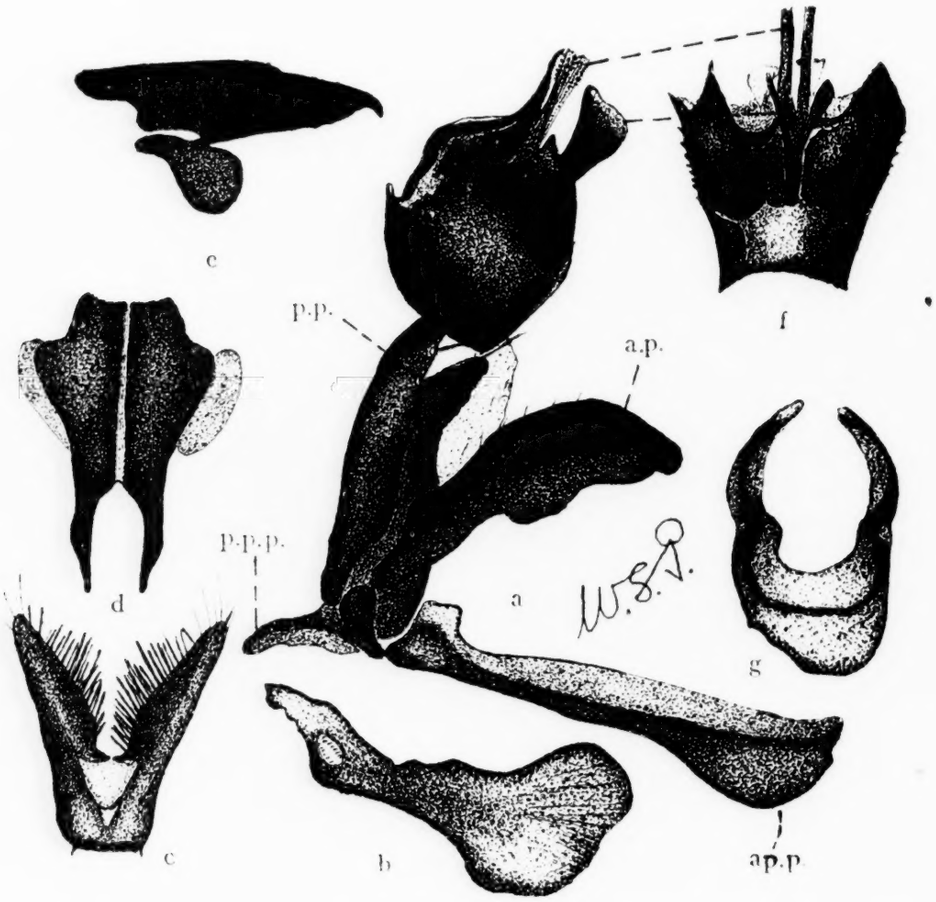


FIG. 5. *a*.—Phallosome and one paramere of *setipennis* in side view ; *b*.—Sperm pump sclerite ; *c*.—Fifth sternum ; *d*.—Ventral view of anal cerci and distal segments of ninth coxites ; *e*.—Lateral view of anal cercus and distal segment of ninth coxite ; *f*.—Dorsal view of end of phallosome ; *g*.—Ninth tergo-sternum.

Sarcophaga striata F. DIAGNOSTIC CHARACTERS OF ADULTS OTHER THAN THOSE OF THE TERMINALIA. ♂. This species has been placed in a separate genus, for which Desvoidy's name *Ravinia* has been used, and there is probably more justification for this action than in other cases where genera have been split off from the major genus *Sarcophaga*. It stood in most lists until recently as *Sarcophaga haematodes* Mg. It is one of the ' red-tailed ' species, and is distinguished from all other European ones known to us by the series of frontal setae being straight. In all others there are three below the level of the base

of the antennae, the two lowest of which are placed outwardly, out of the line of the remainder, making the row appear to curve. In *striata* the lowest seta is missing, and the other two appear in line with the rest of the series. It is a trifling difference but recognizable at a glance, and is a sufficient distinction, at least so far as our British species are concerned. It is a greyer-looking insect than most of our Sarcophagas, and the abdominal pattern is rather more clearly defined and fixed, while, the phallosome also being distinctive, it does seem to stand alone so far as Europe is concerned (although Aldrich quotes several North American species which he thinks are allied to it, and which would have to go into *Ravinia* if that genus be upheld).

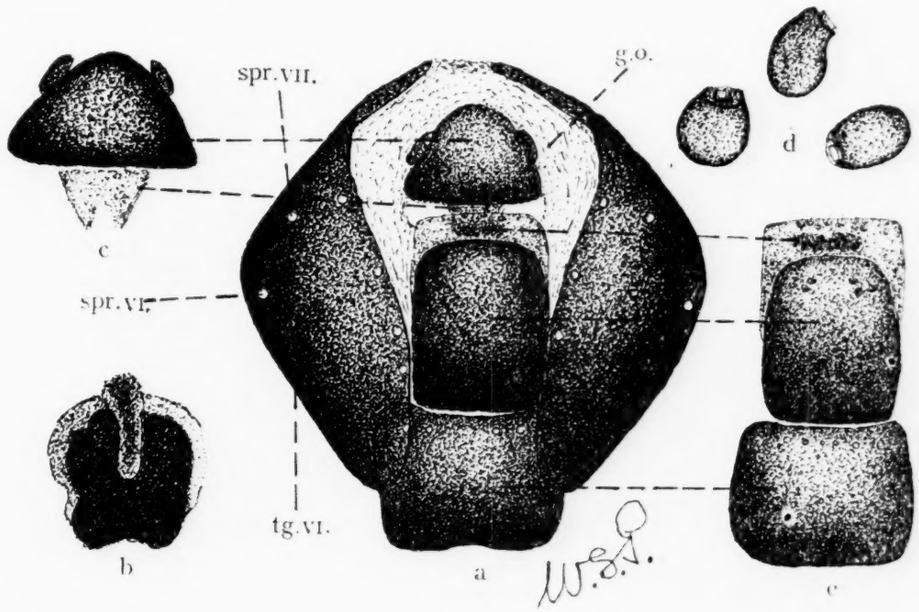


Fig. 6. *a*.—Ventral view of terminalia of ♀ *setipennis* to show diagnostic characters ; *g.o.*—Genital opening ; *spr.vi, spr.vii.*—Sixth, seventh spiracle ; *tg.vi.*—Sixth tergum ; *b*.—Signum ; *c*.—Tenth sternum and anal cerci ; note the small plate attached to the former ; *d*.—Spermathecae ; *e*.—Sixth, seventh, ninth (?) sterna.

Striata is of medium size, usually about 8 mm. long ; frons at narrowest about two-thirds eye-width ; outer verticals missing or but slightly developed ; antennae with third joint quite twice length of second ; genae wide, more than half eye-width, with a number of fine setae. Thorax with three clearly marked, rather brownish stripes ; two or three pairs of large and strong presutural acrs. ; one small pair of prescutellar acrs. ; three postsutural D.C. ; small prealars. Scutellum with no apicals, one pair strong preapicals, one pair strong basals and a pair of weak discals. Abdomen nearly cylindrical, with three lines of fairly distinct dentate markings ; no marginals on second visible segment, and only a weak pair on the third, but a full series of long strong ones on the fourth ; the first genital segment (seventh tergum) also has a full series of short setae,

and is dull and grey, with a reddish margin ; and the second genital (tenth tergum) is entirely red, with no definite setae. No costal thorn ; third costal section about equal to fifth ; no setae on first longitudinal, but a short series on basal portion of third. Sterna with long pilosity, and lamellae of apical sternum with well-defined ' brush ' of small closely set setae. LEG CHAETOTAXY. No long or dense pilosity on any of the legs ; fore femora with a full series of postero-ventral bristles ; one postero-ventral seta on front tibiae ; mid femora with antero-ventral row much reduced, three or four short bristles on basal portion,

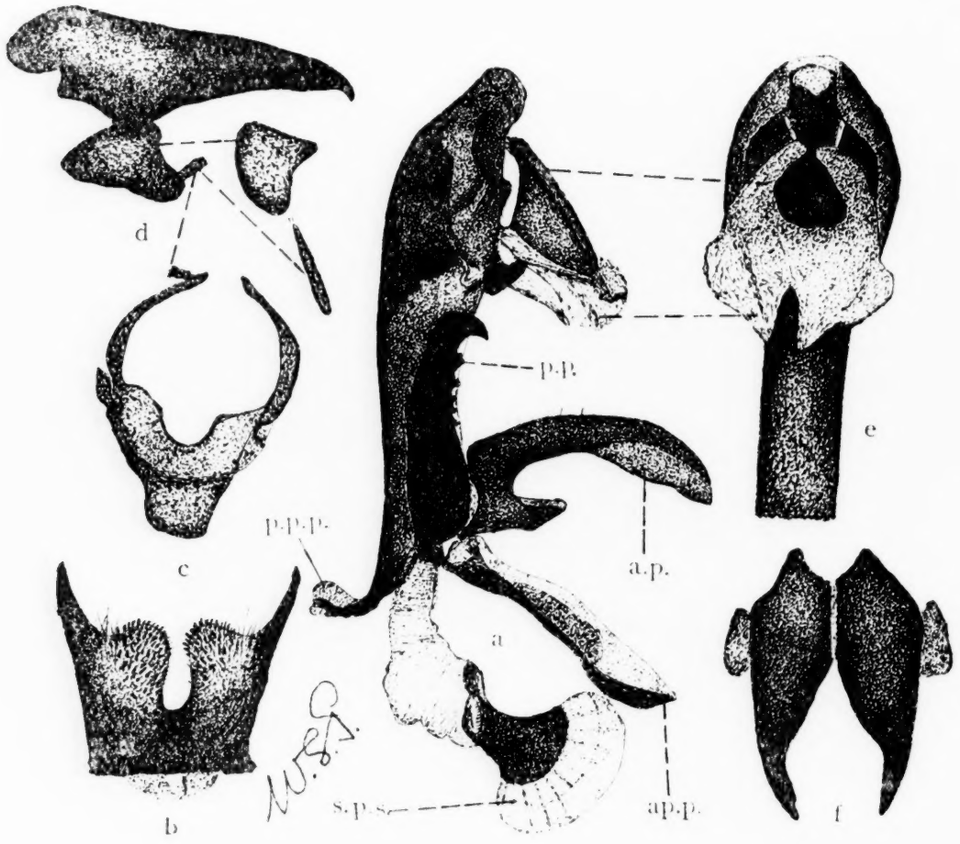


FIG. 7. *a*.—Phallosome and one paramere of *striata* in side view ; lettering as in fig. 1, *a* ; *b*.—Fifth sternum ; *c*.—Ninth tergo-sternum ; *d*.—Lateral view of anal cercus and two parts of ninth coxite, for in this species the proximal segment is a well-developed rod ; *e*.—Dorsal view of end of phallosome ; *f*.—Ventral view of anal cerci and distal segments of ninth coxites.

and a few fine inconspicuous ones near tip ; postero-ventral row with four or five moderately long bristles about middle, and about seven short stout setae at tip forming the so-called ctenidium ; mid tibiae without the usual ventral bristle ; hind femora with an irregular scattered antero-ventral series, and only two strong bristles at base on postero-ventral aspect.

♀. The female is as readily identified as the male, being like it in nearly every respect, and is distinguished from the females of other species by the

same distinguishing characters. The frons is wide, about one-quarter as wide again as an eye ; thoracic scutellars and abdominal chaetotaxy, etc., the same as in the ♂ ; sterna without the long pilosity of the ♂ and, excepting the first, nearly hidden by the overlapping terga ; a row of strong bristles on margin of fifth tergum ; sixth tergum undivided. LEG CHAETOTAXY. Similar to that of the ♂, but no ctenidium, and a few fine hair-like bristles in its place, and the ventral bristle on mid tibiae well developed.

NOTES. This is not one of our common species, but it has been taken by us in a number of places in the south and the Midlands, from Wye, Kent to Cornwall, and up to Yarmouth and Wyre Forest ; Mr. H. Britten has taken it at Birkdale in Lancashire, but it is not yet known from further north. It has

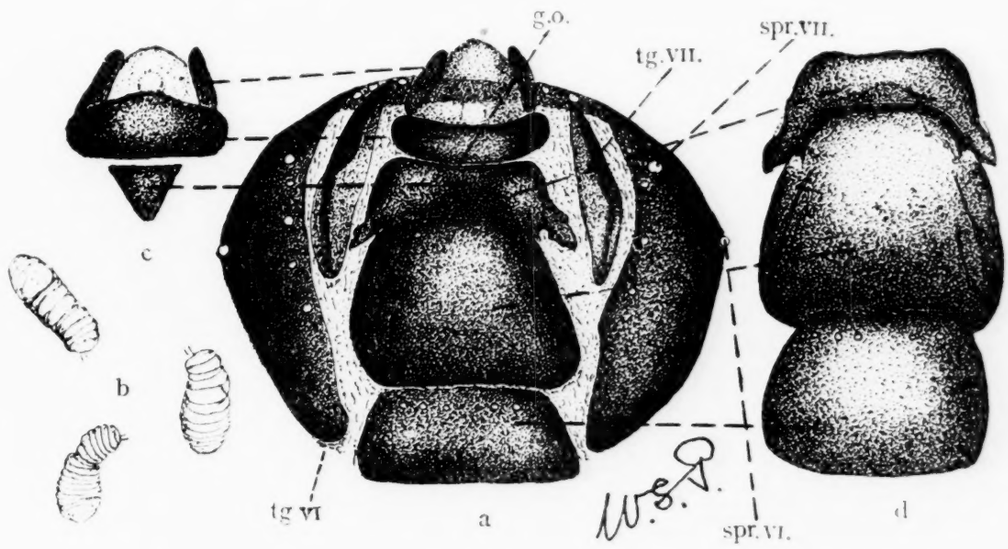


FIG. 8. *a*.—Ventral view of terminalia of ♀ *striata* to show diagnostic characters ; lettering as in fig. 2, *a* ; *b*.—Spermathecae ; *c*.—Tenth sternum and anal cerci ; note the small triangular plate attached to the former ; *d*.—Sixth, seventh, ninth (?) sterna ; note the characteristic shape of the last.

not yet been found anywhere in quantity. Böttcher says that it is widely distributed in the Palaearctic region, and perhaps is nowhere rare ; he also records it from Tunis and northern India, and Lundbeck adds the Canary Islands.

DIAGNOSTIC CHARACTERS OF TERMINALIA. ♂. Fig. 7. In side view each anal cercus (fig. 7, *d*) is a broad plate tapering to the end, which is bent upwards ; the ventral view of the two cerci is shown in fig. 7, *f*. In this species the proximal segment of the ninth coxite is well developed, consisting of a slender rod (fig. 7, *d*) which is loosely attached to the postero-dorsal edge of the distal segment and articulates with the end of the posterior process of the ninth tergo-sternum (fig. 7, *c*). The phallosome (fig. 7, *a*) has a very characteristic appearance in side view, the long straight posterior border ends in the short posterior process, which is bent, suggesting a tail ; the distal end is blunt, and projecting from the

middle of the dorsal surface there is a bilateral semi-membraneous process which is quite characteristic in both lateral and dorsal view (fig. 7, *e*). The posterior part of the paramere (fig. 7, *a*) is short, broad and hooked at the end, and has a row of short spines which may extend almost to the base ; the anterior part (fig. 7, *a*) is a broad bent plate. Sternum 5 (fig. 7, *b*) is short and broad, with a deep emargination, the rounded lobes beset with numerous fine hairs, and produced at the side into a narrow process.

♀. Fig. 8, *a*. Tergum 6 consists of a single plate ; tergum 7 consists of a pair of long, broad plates widely separated. Sternum 6 (fig. 8, *d*) is square, sternum 7 wider and much longer, and attached at its distal end there is a characteristic plate, the sides extending backwards like horns. Sternum 10 (fig. 8, *c*) is a wide narrow plate, with a small triangular plate attached to it. The signum is wanting.

(*To be continued*)

ON THE LIFE-HISTORY OF *LECITHODENDRIUM PYRAMIDUM* LOOSS, 1896, AND ITS DEVELOPMENT FROM A XIPHIDOCERCARIA, *C. PYRAMIDUM* SP. NOV., FROM *MELANIA TUBERCULATA*

BY

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For the past few years, frequent visits have been made to Rashda, a village in the Dakhla Oasis, to investigate the problem of the prevention of schistosomiasis.

Several hundreds of snails were collected from the different springs and streams of the village and were examined for larval trematodes. So far, four different species of cercariae have been found :—

1. *C. pleurolophocerca* Sonsino, 1895.
2. *C. distomatosa* Looss, 1896.
3. A brevifurcate apharyngeal cercaria with eye-spots, described in detail elsewhere (Azim, 1935).
4. A Xiphidocercaria of the 'Virgula' group of Sewell, *C. pyramidum* sp. nov.

The last is a minute cercaria, with a strong tendency to swim towards the surface of the water, which contains the snails. The body measures 210μ in length and 140μ in breadth, and the tail, fully extended, is 110μ in length. The oral sucker is oval, measuring 50 $\times$ 60μ and possessing a highly refractile structure, consisting of two pear-shaped organs communicating with a transverse piece (figs. 2, 3). The fact that it disappears immediately after penetration may throw some light on its function as a support during the process. The absence of this virgula structure from the immature stages is in favour of this view. It has also been noticed that it disappears or degenerates in specimens more than 24 hours old.

The stylet is 18μ in length and has a sharp spine with a circular thickening at the junction of the anterior with the two posterior thirds. Its diameter increases towards the rounded posterior extremity. In the course of this work, I have observed many cercariae retaining the stylets 72 hours after penetration into the intermediate host. This throws some doubt on its value as a penetrating organ, and supports the view of Faust on this subject.

The mouth is subterminal and is surrounded by the virgula organ, the transverse part of which passes dorsal to it. The pharynx is a cellular structure below the oral sucker. No oesophagus or intestinal caeca were observed.

The stylet glands are three on either side. The anterior is the smallest

of the three and lies lateral to the pharynx. The other two lie lateral to the ventral sucker and are more intensely granulated than the anterior gland. From each gland, a separate duct travels anteriorly and dorsally, to pierce the parenchyma of the anterior organ and to open near the base of the stylet.

The ventral sucker is 30μ in diameter.

The excretory system consists of a bicornuate bladder lying at the posterior extremity of the body. From the antero-lateral margin of each cornua, arises the main excretory duct, which passes anteriorly and gives off a branch, which travels downwards and then continues its course to the vicinity of the ventral sucker, where it divides into three subsidiary branches. Owing to the presence of numerous cystogenous glands in the parenchyma of the body, I have not been able to trace the finer ramifications and the flame-cells.

Development of the cercaria takes place in minute sporocysts measuring 250μ × 300μ. They contain cercariae in different stages of development, together with germinal cells (fig. 1).

Encystation. After a long series of experiments, it was found that encystation took place in anopheline mosquito larvae ; *A. pharoensis* and *A. multicolor* served equally well for this purpose. Other culicine larvae were not suitable. Whether this discrimination had anything to do with the mode of feeding (the anophelines being surface feeders) or with the composition of the alimentary juices remained to be cleared up.

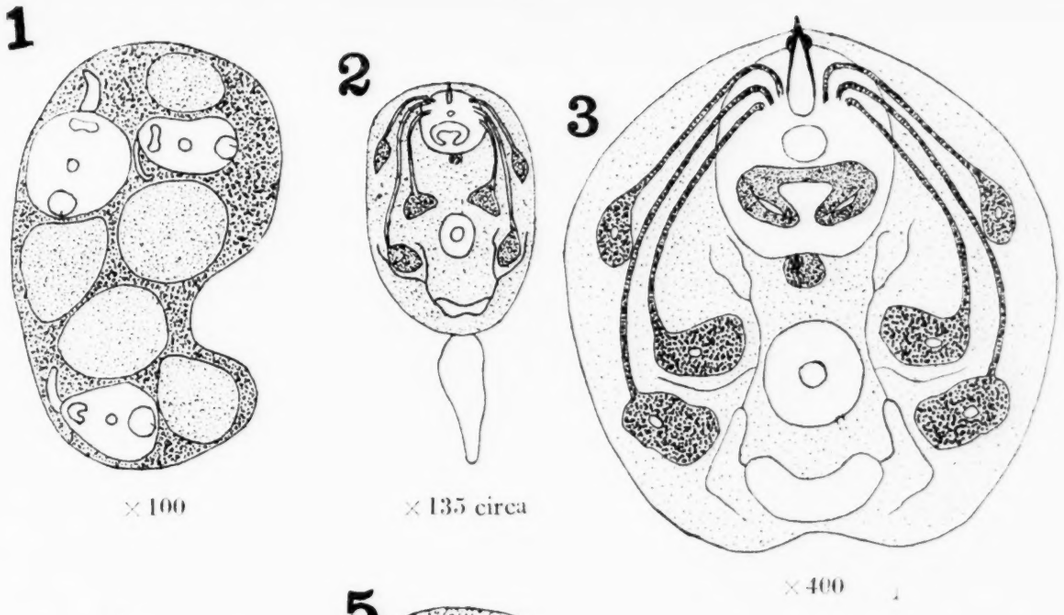
When put into the same container as the cercariae, the larvae become suddenly active and pursue the cercariae. Four hours later, dissection shows the tailless cercariae in the alimentary canal and afterwards in the thoracic and abdominal segments. Heavy infections are very deleterious to the larvae and cause their death. Moderate infections, however, seem to affect their vitality and to retard their growth considerably.

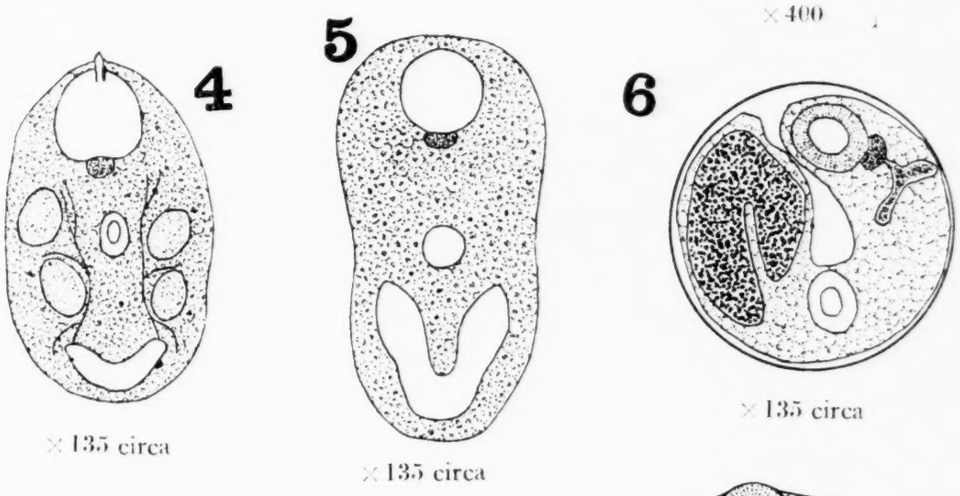
Having reached the thoracic and abdominal segments, they remain motile for 5–7 days before encystation. The first change to take place is the disappearance of the stylet glands and the virgula organ. The outline of the oral sucker is fainter and the cystogenous glands are more conspicuous. The excretory vesicle assumes a V-shape, the limbs reaching the posterior border of the ventral sucker (figs. 4, 5).

The cysts (fig. 6) are globular and measure 240μ in diameter. The protoplasm of the metacercaria inside is now clear, the two suckers are vivid and the pharynx and two short intestinal caeca begin to make their appearance. The excretory vesicle is full of dark granules. No trace of the genitalia was seen. The metamorphosis of the larva, from the moment of its penetration into the second intermediate host to its maturity inside the cyst, takes 2–3 weeks according to the temperature of the atmosphere (fig. 7).

The cysts were noted to pass from the larva to the pupa and ultimately to the imago stages of the mosquito with no apparent change.

The character and morphology of the parasite and the structure of the





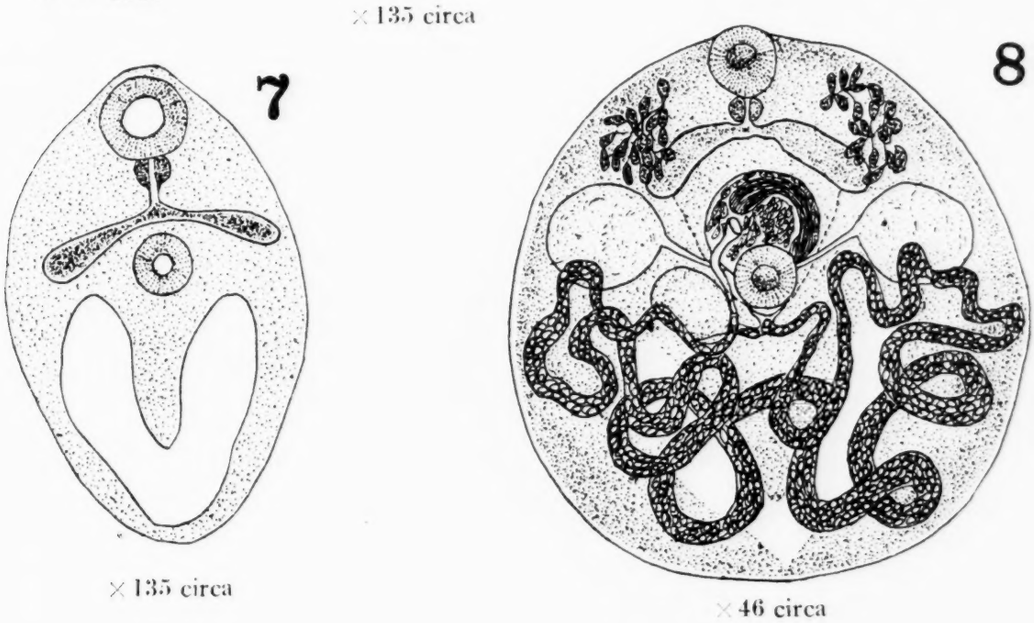


FIG. 1. A sporocyst enclosing different stages of development.
FIG. 2. A mature cercaria from the *Melania tuberculata*.
FIG. 3. The body of a mature cercaria showing the stylet, stylet glands and the excretory system.
FIG. 4. The body of the cercaria after penetration into the anopheline larva.
FIG. 5. The cercaria 9 days after penetration.
FIG. 6. A cyst from the thorax of *A. pharoensis* 12 days after penetration.
FIG. 7. The metacercaria liberated from the cyst.
FIG. 8. An adult trematode from the small intestine of a bat, *Vespertilio euryale*.

excretory vesicle suggest that the adult trematode may belong to the subfamily Lecithodendrinae.

As the Egyptian members of this subfamily were recorded from insectivora, e.g., chameleons and bats, search was made for these in the village. The former were absent, but the latter were present in large numbers. *Vesperugo kuhli* and *Rhinolphus euryale* were the only species met with.

Bats have been known to feed on mosquitoes on the wing to such an extent that they were considered as a control measure in combating them.

Dissection revealed large numbers of *Lecithodendrium pyramidum* Looss, 1896 (fig. 8), in the alimentary canal of both species. It was also the only representative of this genus found in all the bats dissected.

It was found impossible to carry out feeding experiments to prove that the cysts developing in the mosquitoes would ultimately develop into the trematodes seen in the bats. Experience had shown that the bats were very difficult to keep in captivity.

The presence of only one Xiphidocercaria in the large numbers of snails collected, the presence of large numbers of anopheline mosquitoes, larvae and adults, the existence of insectivorous bats in the same locality, harbouring only one species of *Lecithodendrium*, together with the striking similarity between the excretory system in both larvae and adults, justify the conclusion that the cercaria described above is the larva of *Lecithodendrium pyramidum* Looss, 1896.

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PLATE XI

EXPLANATION OF PLATE XI

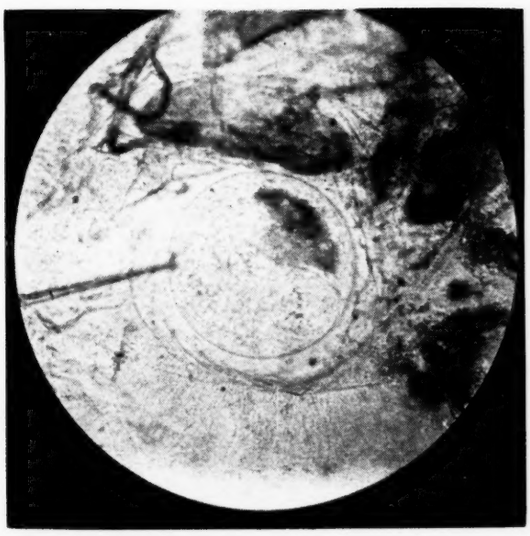
Fig. 1. Microphotograph showing cysts of *Lecithodendrium pyramidum* in the tissues of *A. multicolor* larva.

Fig. 2. Microphotograph showing a mature cyst with a V-shaped excretory vesicle.

Fig. 3. Microphotograph showing cysts pressed out of the segments of *A. multicolor* imago.

PLATE XI







H. R. Grubb, Ltd., Poplar Walk, Croydon

A SIMPLE METHOD OF PLOTTING LOGARITHMIC GROWTH-CURVES OF PROTOZOAL CULTURES

BY

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(*Received for publication* 18 *June*, 1936)

In attempting to plot logarithmic curves of the growth of intestinal protozoa in cultures I have met with several difficulties :—

1. After much searching I obtained semi-logarithmic paper, on which the ordinates ran from 1 to 100 only, so that if I wanted to plot a growth from 1 to 20,000 (as occurs in *Trichomonas* cultures), I had to paste together three sheets of paper.

2. Whereas on arithmetically ruled paper one can use the scale that is most convenient, on logarithmic paper one is bound to use the scale that the printer has fixed. In my case a 1 : 10 ratio covered 10 cm. on the ordinate axis ; hence, a curve of a growth from 1 to 10,000 had a height of 40 cm.

3. If one division on the abscissal axis (1 cm.) was used for one time-unit (one day), the curves became so steep that they failed in their aim to give a good visual impression of differences in growth-ratio. One day had to be made 3 cm. on the abscissal axis, which resulted in very broad sheets of paper.

4. The handling of these large papers is inconvenient.

5. Logarithmic paper is rather expensive, especially if used by square meters.

6. For reproduction the diagrams must be remodelled.

These drawbacks can be partly overcome by using paper ruled in square millimetres, by looking up in a table the logarithms of the figures to be plotted, and by applying a scale which, as far as form and dimensions are concerned, results in a becoming curve. Now, looking up logarithms in a table with, say, five decimals takes up a considerable time. Moreover, it is laborious to fit the logarithms, as found, into the scale which one wishes to use on the ordinate axis.

For my purpose it seemed convenient if 1 cm. of the ordinate axis covered a 1 : 2 ratio and 1 cm. of the abscissal axis one day. In order to plot the logarithms on the square-ruled paper without any further calculation, I have composed a very simple table of logarithms with 2 as a base. The logarithms in this table are rounded off to half the first decimal of the mantissa, i.e., $\frac{1}{2}$ a millimetre on the ordinate axis. The maximal error that is made in this way is $\frac{1}{4}$ millimetre or $2^{0.025} - 1 = 0.017 = 1.7$ per cent. The whole table (page 359) does not cover more than one page, and the logarithms wanted can be found at a

glance. It is obvious that the form of the curve is the same as when common decimal logarithms are used.

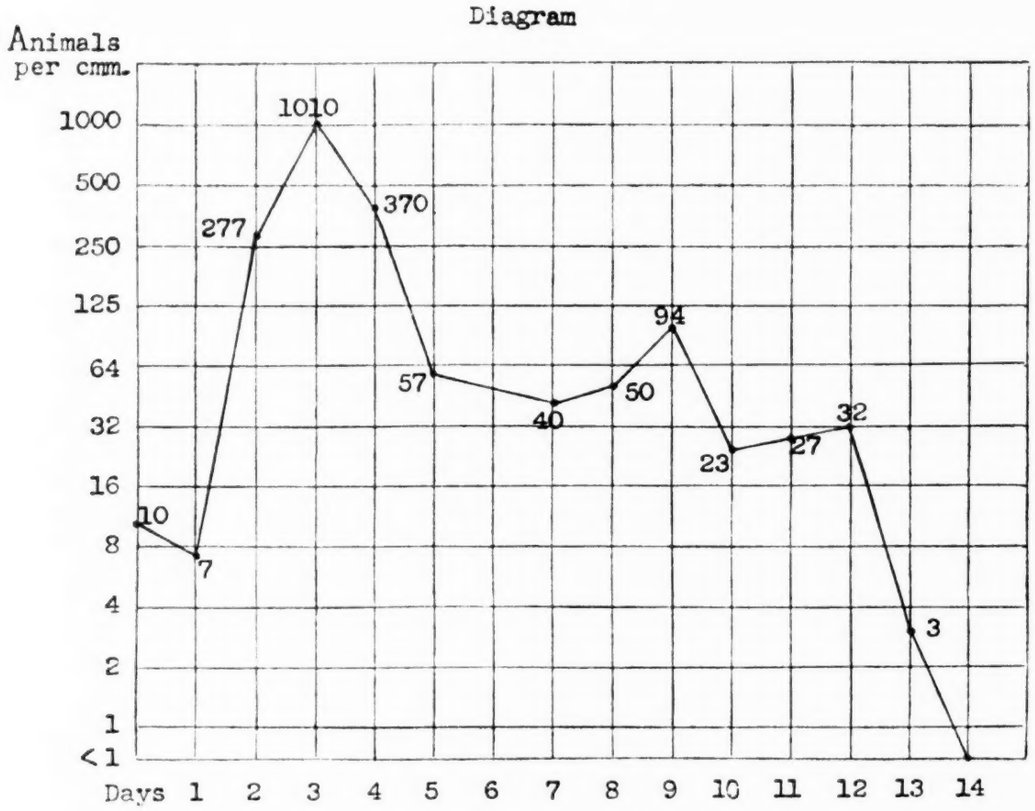
The logarithms with 2 as a base can be deduced in a simple way from the common decimal logarithms :—

$$\log_{10} 2 = 0{\cdot}30103, \text{ or rounded off } 0{\cdot}3$$

$$\text{so } \log_2 n = \frac{1}{0{\cdot}3} \log_{10} \qquad n = \frac{10}{3} \log_{10} n,$$

or, to convert decimal logarithms into those with 2 as a base, multiply by 10 and divide the product by 3. The rounding off of 0·30103 into 0·3 gives a very small error, as $10^{0{\cdot}3} = 1{\cdot}9953$. In this way the table is composed. It gives at once the height in cm. above the abscissal axis of the figure which one wants to plot.

As an example I give the growth of a *Trichomonas* culture in a diagram made in this way.



Culture of *Trichomonas hominis* in serum 1 : 15, Ringer.

Of course the table can be used for any purpose, provided no great accuracy is required, e.g., to plot logarithmically the agglutination titres of blood-serum in the course of a disease.

Table of logarithms with 2 as base

1·0 – 0·0	5·6 – 2·5	11 – 3·45	56 – 5·85	110 – 6·8	560 – 9·15
1·1 – 0·15	5·7 – 2·5	12 – 3·6	57 – 5·85	120 – 6·95	570 – 9·2
1·2 – 0·25	5·8 – 2·55	13 – 3·7	58 – 5·9	130 – 7·05	580 – 9·2
1·3 – 0·4	5·9 – 2·55	14 – 3·8	59 – 5·9	140 – 7·15	590 – 9·25
1·4 – 0·5	6·0 – 2·6	15 – 3·9	60 – 5·95	150 – 7·25	600 – 9·3
1·5 – 0·6	6·1 – 2·6	16 – 4	61 – 5·95	160 – 7·35	610 – 9·3
1·6 – 0·7	6·2 – 2·65	17 – 4·1	62 – 5·95	170 – 7·45	620 – 9·3
1·7 – 0·75	6·3 – 2·65	18 – 4·2	63 – 6	180 – 7·5	630 – 9·35
1·8 – 0·85	6·4 – 2·7	19 – 4·25	64 – 6	190 – 7·6	640 – 9·35
1·9 – 0·95	6·5 – 2·7	20 – 4·35	65 – 6·05	200 – 7·65	650 – 9·4
2·0 – 1	6·6 – 2·75	21 – 4·4	66 – 6·05	210 – 7·75	660 – 9·4
2·1 – 1·05	6·7 – 2·75	22 – 4·45	67 – 6·1	220 – 7·8	670 – 9·4
2·2 – 1·15	6·8 – 2·75	23 – 4·55	68 – 6·1	230 – 7·85	680 – 9·45
2·3 – 1·2	6·9 – 2·8	24 – 4·6	69 – 6·15	240 – 7·95	690 – 9·45
2·4 – 1·25	7·0 – 2·8	25 – 4·65	70 – 6·15	250 – 8	700 – 9·5
2·5 – 1·35	7·1 – 2·85	26 – 4·7	71 – 6·15	260 – 8·05	710 – 9·5
2·6 – 1·4	7·2 – 2·85	27 – 4·75	72 – 6·2	270 – 8·1	720 – 9·5
2·7 – 1·45	7·3 – 2·85	28 – 4·8	73 – 6·2	280 – 8·15	730 – 9·55
2·8 – 1·5	7·4 – 2·9	29 – 4·85	74 – 6·25	290 – 8·2	740 – 9·55
2·9 – 1·55	7·5 – 2·9	30 – 4·9	75 – 6·25	300 – 8·25	750 – 9·6
3·0 – 1·6	7·6 – 2·95	31 – 4·95	76 – 6·25	310 – 8·3	760 – 9·6
3·1 – 1·65	7·7 – 2·95	32 – 5	77 – 6·3	320 – 8·35	770 – 9·6
3·2 – 1·7	7·8 – 2·95	33 – 5·05	78 – 6·3	330 – 8·4	780 – 9·65
3·3 – 1·75	7·9 – 3	34 – 5·1	79 – 6·35	340 – 8·45	790 – 9·65
3·4 – 1·75	8·0 – 3	35 – 5·15	80 – 6·35	350 – 8·5	800 – 9·7
3·5 – 1·8	8·1 – 3·05	36 – 5·2	81 – 6·35	360 – 8·5	810 – 9·7
3·6 – 1·85	8·2 – 3·05	37 – 5·25	82 – 6·4	370 – 8·55	820 – 9·7
3·7 – 1·9	8·3 – 3·05	38 – 5·25	83 – 6·4	380 – 8·6	830 – 9·75
3·8 – 1·95	8·4 – 3·1	39 – 5·3	84 – 6·4	390 – 8·65	840 – 9·75
3·9 – 1·95	8·5 – 3·1	40 – 5·35	85 – 6·45	400 – 8·65	850 – 9·8
4·0 – 2	8·6 – 3·1	41 – 5·35	86 – 6·45	410 – 8·7	860 – 9·8
4·1 – 2·05	8·7 – 3·15	42 – 5·4	87 – 6·45	420 – 8·75	870 – 9·8
4·2 – 2·1	8·8 – 3·15	43 – 5·45	88 – 6·5	430 – 8·8	880 – 9·8
4·3 – 2·1	8·9 – 3·15	44 – 5·5	89 – 6·5	440 – 8·8	890 – 9·85
4·4 – 2·15	9·0 – 3·2	45 – 5·5	90 – 6·5	450 – 8·85	900 – 9·85
4·5 – 2·2	9·1 – 3·2	46 – 5·55	91 – 6·55	460 – 8·9	910 – 9·85
4·6 – 2·2	9·2 – 3·2	47 – 5·55	92 – 6·55	470 – 8·9	920 – 9·9
4·7 – 2·25	9·3 – 3·25	48 – 5·6	93 – 6·55	480 – 8·95	930 – 9·9
4·8 – 2·25	9·4 – 3·25	49 – 5·65	94 – 6·6	490 – 8·95	940 – 9·9
4·9 – 2·3	9·5 – 3·25	50 – 5·65	95 – 6·6	500 – 9	950 – 9·95
5·0 – 2·35	9·6 – 3·25	51 – 5·7	96 – 6·6	510 – 9·05	960 – 9·95
5·1 – 2·35	9·7 – 3·3	52 – 5·7	97 – 6·6	520 – 9·05	970 – 9·95
5·2 – 2·4	9·8 – 3·3	53 – 5·75	98 – 6·65	530 – 9·1	980 – 9·95
5·3 – 2·4	9·9 – 3·35	54 – 5·75	99 – 6·65	540 – 9·1	990 – 10·0
5·4 – 2·45	10·0 – 3·35	55 – 5·8	100 – 6·65	550 – 9·15	1000 – 10·0
5·5 – 2·45					

ANOPHELES FUNESTUS (GILES) AS A 'DOMESTIC' BREEDER*

BY

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(*Received for publication* 4 *August*, 1936)

This note concerns the behaviour of *A. funestus* Giles, type form, at Malindi, a small town of some 750 dwellings, situated on the east coast of Kenya, about 80 miles north of Mombasa.

Description of the town

Like Lamu and many other coast towns, Malindi was at one time a thriving Arab settlement. It is now an aggregation of historic Portuguese and Arab ruins, more recent Arab houses, mosques and wells rapidly approaching the historic state, and still more recent mud-and-pole huts of the local Africans.

People

The population is made up of Arabs, Swahili, Giriama and other coast tribes, mixed and unmixed, and Indians.

Activities are not overdone. Raw materials for trade, consisting chiefly of maize and cotton, are produced mainly by the Giriama of the surrounding districts. Trading is carried on by Arabs and Indians. A cotton ginnery is kept busy for a good part of the year, dealing with the local crop. The fishing industry, which is capable of considerable expansion, is the effort of a few of the more energetic coastal Africans.

In such a community, civic pride is not obvious. Life is sustained with a minimum of individual effort : a ' public ' spirit has yet to be developed.

Efforts at sanitation are being made by the local authority. Many of the old houses possess inside pit-latrines ; but Africa or the ocean edge serves for perhaps a majority of the inhabitants.

Water-supplies

Water for all purposes is obtained from wells, except during the wet season when it is more easily obtained by the less fastidious from surface pools or by collecting from the roofs. Most of the wells were constructed many years ago by the old Arab community, and are now in a state of serious dilapidation. The majority vary from 15 to 30 feet in depth, and contain a foot or two depth of brackish water. Some are situated inside the houses but most of them are outside. Not one is provided with a cover of any sort.

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*Published by permission of the Director of Medical Services, Kenya.

The people draw water in any suitable bucket, tin or similar vessel, and store it in larger vessels—drums, barrels, pots and tins or tanks—in their houses. In those houses blessed with pit-latrines, there are stone or cement tanks, or large drums, with a capacity of many gallons, in which water is stored in quantity for washing after defaecation.

Malaria

The population suffers from ailments common to most African communities. Malaria is the chief of these. In the following Table is shown the average monthly malaria cases treated at the small Government hospital, together with average monthly rainfall. As is usual where only a clinical diagnosis is possible, these figures probably include ' fevers ' that may not be due to malaria ; but these will not affect the picture to any great extent.

TABLE

Showing the average monthly numbers of malaria cases treated in Malindi Hospital over the last 11 years, and the average monthly rainfall over 40 years

	Jan.	Feb.	March	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Malaria ...	85	65	58	64	64	65	65	68	65	67	69	67
Rainfall in inches ...	0·49	0·29	1·26	6·03	12·73	6·23	3·68	1·97	1·77	2·31	2·22	0·93

In 1929 the spleen rate amongst the Arab school-boys was 34·3 per cent. The same school showed a rate of 21 per cent. in 1934, and the Indian boys' school one of 26 per cent. in September, 1935. In a collection of 47 blood slides taken from the Arab school-boys in June, 1935, eight showed sub-tertian and two benign tertian parasites (23 per cent. infection). These figures, though crude, indicate a considerable amount of infection.

From the records available there appears to be very little seasonal fluctuation in the incidence of infection, but rather a steady rate in all months of the year. This suggested that *A. gambiae*, which in other parts of Kenya varies usually in density with the rainfall, has little if anything to do with Malindi malaria. And this suggestion receives support from the apparent scarcity of *A. gambiae* breeding grounds even during the wet season.

Breeding places of A. funestus

(*a*) *Domestic water-containers.* Our first brief mosquito search was carried out in 1927. We recorded a few breeding places, producing small numbers of *A. gambiae*, *A. mauritianus*, *A. squamosus* and others. A few house searches

produced one or two *A. funestus* adults, and small numbers of larvae of this species in domestic water-containers. Not until the end of 1933 were we able to resume rather more intensive investigations. Since then we have consistently recorded *A. funestus* larvae in wells and in domestic water-containers of all kinds. A sample of our more recent findings will indicate the intensity of production.

In April, 1936, some 120 houses and mosques were searched. Sixty of these had cement or stone tanks containing water, and of these 10 produced larvae of *A. funestus*. Of the numerous pots, tins and barrels, three produced larvae of this species.

During May, about 500 searches were made in houses and mosques, and 24 collections of *A. funestus* larvae were made—14 in tanks and 10 in pots, tins and similar small containers. Larvae in all stages of growth occurred.

Probably these figures do not indicate the true incidence of this species. The houses are so dark, and *A. funestus* is so notoriously elusive, that electric torches have to be used in all searches for larvae. Moreover, an attempt at control has been going on for some time, and African assistants employed for this duty have orders to empty or oil all domestic vessels containing larvae of any kind. Scientific records therefore suffer.

(*b*) *Wells.* There are some 90 wells in the town. During these same two months, 177 routine inspections have been made of these wells. These produced *A. funestus* larvae, of all stages, on 62 occasions. The captures were made with the aid of a special well ' dipper,' but it is probable that many infestations were missed.

A. gambiae. It may be of interest to record here that we find *A. gambiae* occasionally breeding in wells, and more rarely in domestic vessels.

Searches for adults

Our ordinary methods of searching for adults in houses have given unsatisfactory results. Houses are constructed of mud and poles, with thatches of plaited palm leaves. Irregularities in the wall surfaces and the comparative height of the unceilinged 'rooms' make the spotting of small anophelines like *A. funestus* extremely difficult. Our catches of this species are so small that in the absence of our larval records we should be quite unable to account for more than a negligible amount of malaria. We catch reasonably large numbers of *A. aegypti* adults, though its larvae are not very much commoner than those of *A. funestus*. The difference in choice of resting sites must account for this.

Probable sources of A. funestus

It is certain that many of the larvae found in containers inside the houses are brought in with the daily supplies of water from the wells. We have searched vessels containing newly drawn water at the well side, and found many to contain larvae. But where water is stored in quantity and for indefinite periods, as it is in house and mosque tanks and barrels, it is more than probable that *A. funestus*

females make use of such water for ovipositing, and that continuous breeding, therefore, goes on in houses.

Control

This is likely to be somewhat difficult and protracted. Wells are seriously to blame. They cannot be abolished until an alternate supply of water is available—and there is little promise of this. But they might be covered. We are relying upon the efforts of a small local fish, *Telapia mozambicus*, to reduce well-infestations until some permanent structural improvements can be provided.

Fish will probably constitute our only measure for some long time against breeding in mosque tanks, the water of which is used for ablution. Here we have to feel our way slowly, lest we commit some outrage on religious observance or sentiment.

In houses we are more at home. Nothing but persistent drastic measures will serve, and these must be applied over a very long period. They have been started. All water containing larvae of any species of mosquito is thrown away or oiled. The people are already exhibiting less resentment towards those who apply this rough treatment.

TRYPANOLYSIS *IN VITRO* BY MOUSE IMMUNE SERUM*

BY

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AND

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(From the Liverpool School of Tropical Medicine)

(Received for publication 8 September, 1936)

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INTRODUCTION

Since the early work of Schilling (1902), it has been repeatedly confirmed, by numerous investigators, that the serum of certain animals infected with pathogenic trypanosomes may have a pronounced lytic action against the infecting agent. The accounts of this phenomenon in the literature deal almost exclusively with the serum of animals harbouring a chronic, or relapsing, type of infection, such as commonly develops in guinea-pigs and larger animals. In the case of mice, it is, of course, well known that untreated infections by trypanosomes of the type of *T. equinum* and *T. brucei*, which have been constantly maintained in this host, characteristically run an unremitting, rapidly fatal course. Now it might be expected that, as the natural crisis occurring in infections of larger animals is associated with the appearance of trypanolysins demonstrable *in vitro*, so in mouse infections of the type mentioned, the crisis artificially produced by drug treatment might equally be associated with the appearance of trypanolysins

* This work was assisted by a grant from the Chemotherapy Committee of the Medical Research Council, and the writers are indebted to Professor Warrington Yorke, F.R.S., for criticism and advice.

readily demonstrable *in vitro*. This is perhaps rendered all the more likely by the fact, first established by Ehrlich and Shiga (1904), that efficient drug treatment of a mouse infection is followed by a definite degree of immunity against the infecting strain ; furthermore, Taliaferro and Johnson (1926) have shown a curative property of mouse immune serum when injected into other mice infected with the homologous trypanosome (' *in vivo* trypanolysis '). However, very scant attention has hitherto been paid to the demonstration of trypanolysis *in vitro* by the serum of immune mice, and such attempts as are reported appear to have had negative or otherwise unsatisfactory results.

Rosenthal (1913) declares that trypanolysis *in vitro* by mouse immune serum either does not occur at all or does so only to a very slight extent. In the belief that complement is necessary for trypanolysis by an immune serum (see also Levaditi and Mutermilch, 1909 ; Levaditi and McIntosh, 1910 ; Kligler, 1931), Rosenthal relates the apparent failure of mouse immune serum to exhibit trypanolysis *in vitro* to the fact (for which he quotes Ritz, 1911) that the serum obtained from mouse blood does not contain the full complement complex, being deficient in the end-piece fraction. Kokawa (1928), likewise, declares that trypanolysis *in vitro* by mouse immune serum is scarcely demonstrable, on account of the lack of end-piece and third component in normal mouse serum. Schilling and Neumann (1932), discussing the relative value of various methods for immunological differentiation of trypanosome strains, compare *in vitro* trypanolytic studies with the procedure of mixing immune serum and trypanosomes for 1 hour at room temperature, inoculating into a mouse, and observing whether or not an infection becomes established in the mouse. They describe experimental work in this connection with immune serum from a variety of host-species, and state that, with mouse immune serum, the *in vitro* method (with or without complement, at room or incubation temperature) is unsatisfactory and might be completely negative in a case where the other method gives a completely positive result.

In the course of the experimental work to be described in this contribution, we have had no difficulty in eliciting lysis of the homologous strain, by the serum of mice immune (or relatively immune) to various trypanosomes at our disposal, viz. :—*T. rhodesiense*, *T. equinum*, *T. equiperdum* and two strains of *T. brucei*, all of which have been maintained in mice, in unbroken passage, for considerable periods.* While the trypanolytic process is rapidly effective in

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* The periods during which the strains have been maintained in mice are as follows :—

(*a*)	*T. rhodesiense*	13 years.
(*b*)	*T. equinum*	3 years.
(*c*)	*T. equiperdum*	8 years.
(*d*)	*T. brucei* ; strain to be referred to in the present communication as the parent *T. brucei* strain ...	4 years.
(*e*)	*T. brucei* ; strain to be referred to as the fly *T. brucei* strain, having been derived from the parent strain after passage through guinea-pigs and tsetse-fly...	3 years.

the presence of added haemolytic complement, it has been found also to occur, although more slowly, in its entire absence. The rôle of complement in this matter has been subjected to some experimental analysis. We have also made a number of observations upon the influence of various factors on the trypanolytic titre of the immune serum, viz. :—(*a*) time since treatment, (*b*) degree of infection at the time of treatment, and (*c*) size of dose.

II. IMMUNITY OF TREATED MICE TO REINFECTION BY THE HOMOLOGOUS STRAIN

As a preliminary to the actual *in vitro* investigations, we set out to re-examine the question of the immunity of cured mice. Groups of mice infected with each of the five strains mentioned above received curative doses of 0·08 mgm. or 0·16 mgm. halarsol per 20 gm. mouse. At varying intervals after the blood had been cleared of trypanosomes by this treatment, attempts were made to reinfect each of the mice with the homologous strain, by intraperitoneal inoculation of a small amount of infected blood in sodium citrate solution. In all, 38 mice were examined in this way, and each was found to be either completely, or relatively, immune (i.e., in the latter case, was reinfected, but with a prolonged incubation period). In Table I are given some of the results of these tests ; for economy of space, we have included in this Table only the records of those mice in which the reinoculations were attempted more than 40 days after treatment, since the duration of immunity in these cases has a special interest, to which further attention will be drawn below. The mice which were tested less than 40 days after treatment (in this group all 5 strains were examined) gave results strictly comparable with those shown in the Table. As may be seen, the results bear out the statement of von Jancsó and von Jancsó (1934) that 'delayed development, rather than complete failure, of the second infection is to be observed especially when the second injection is a heavy one.' Thus, summarizing the whole series of 38 mice :—

25 mice reinoculated with 8,000 trypanosomes—
- 20 failed to become infected ;
- 5 showed a prolonged incubation period.

13 mice reinoculated with 160,000 trypanosomes—
- 2 failed to become infected ;
- 11 showed a prolonged incubation period.

A further point of interest, particularly exemplified by mice 158D and 158E, is the feature already pointed out by Franke (1905) and also by von Jancsó and von Jancsó (1934), that, where the reinfection does succeed, it is not unusual to encounter a number-crisis before the animal finally succumbs, although in ordinary infections of mice by such strains a crisis practically never occurs.

The most unexpected feature presented by the above observations, however, is the very long duration of the immunity. Table I shows that this may still be

TABLE I.—Showing immunity, or relative immunity, of treated

Approximate no. of trypanosomes injected		Strain used	Mouse no.	Interval between treatment of original infection and test-inoculation	Result						
					1	2	3	4	5	6	7
8,000	Experimental	*T. rhodesiense*	379 D	200 days		—	—	—	—	—	—
			379 B	180 ,,		—	—	—	—		—
			592 E	132 ,,		—	—	—	—		—
			434 B	105 ,,		—	—	—	—	—	—
			591 B	69 ,,		—	—	—	—	—	—
			591 D	69 ,,		—	—	—	—	—	—
		T. brucei fly strain	159 A	239 ,,	—	—	—	—		—	—
			159 C	239 ,,	—	—	—	—		—	—
			158 C	144 ,,	—	—	—	—		—	—
			158 D	144 ,,	—	—	—	—		—	—
			158 E	144 ,,	—	—	—	—		—	—
			710 C	43 ,,	—	—	—	—		—	—
			710 D	43 ,,	—	—	—	—		—	—
		T. brucei parent strain	854 E	74 ,,		—	—	—	—		—
		T. equiperdum	845 B	77 ,,		—	—	—	—		—
	Control	*T. rhodesiense*	896 C	...		—	10	+++ D			
		T. brucei, fly strain	938 A	...	—	$\frac{1}{500}$	20	+++ D			
		T. brucei parent strain	943 A	...		$\frac{1}{10}$	10	++	D		
		T. equiperdum	939 B	...		2	+	D			
160,000	Experimental	*T. rhodesiense*	379 A	110 days	—	—	—	$\frac{1}{20}$		+++	D
			432 D	98 ,,	—	—	$\frac{1}{?}$	$\frac{1}{1000}$		20	++
			433 C	98 ,,	—	—	—	$\frac{1}{20}$		+++	D
			587 E	62 ,,	—	—	—	—		+	D
			589 D	62 ,,	—	—	2	+		D	
			591 A	62 ,,	—	—	—	$\frac{1}{50}$		+	D
	Control	*T. rhodesiense*	886 A	...	$\frac{1}{2}$	+	D				
			886 B	...	$\frac{1}{10}$	5	+	D			

+ = More than 20 parasites per field. ++, +++ = Parasites swarming. D = Dead.

mice to reinfection by the homologous strain

of test-inoculation (trypanosomes per field, oc. 4 ; obj. $\frac{1}{8}$th)

					Days after test-inoculation													
8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26–60*
	—	—	—	—	—	—		—	—	—	—	—	—		—	—	—	—
—	—	—	—	—		—	—	—	—	—	—		—	—	—	—	—	—
—	—	—	—	—		—	—	—	—	—	—		—	—	—	—	—	—
	—	—	—	—	—	—		—	—	—	—	—	—		—	—	—	—
	—	—	—	—	—	—		—	—	—	—	—	—		—	—	—	—
	—	—	—	—	—	—		—	—	—	—	—	—		—	—	—	—
—	—	—	—		—	—	—	—	—	—		—	—	—	—	—	—	—
—	—	—	—		—	—	—	—	—	—		—	—	—	—	—	—	—
—	—	—	—		—	—	—	—	—	—		—	—	—	—	—	—	—
—	—	$\frac{1}{20}$	10		++	+++	+++	1	—	—		+	D					
—	—	$\frac{1}{50}$	$\frac{1}{2}$		++	+++	+++	$\frac{1}{10}$	$\frac{1}{30}$	$\frac{1}{20}$		+	++	+++	+++	+++	D	
—	—	—	—		—	—	—	—	—	—		—	—	—	—	—	—	—
—	—	—	—		—	—	—	—	—	—		—	—	—	—	—	—	—
—	—	—	—	—		—	—	—	—	—	—		—	—	—	—	—	—
—	—	—	—	—		—	—	—	—	—	—		—	—	—	—	—	—

D

* Between the 26th and 60th days examinations were made every two days.

absolute for at least 8 months (239 days) in the case of the fly strain of *T. brucei*, $6\frac{1}{2}$ months (200 days) for *T. rhodesiense*, and $2\frac{1}{2}$ months each (77 and 74 days respectively) for *T. equiperdum* and the parent *T. brucei* strain. These periods are strikingly longer than one would be led to expect by reference to the literature, even allowing for the fact that, as Browning (1927) points out, the immunity response following treatment by the same agent may vary markedly in degree with different strains of the same trypanosome. Ehrlich and Shiga (1904) gave 20–30 days as the duration of immunity to *T. equinum* in mice after treatment of infections by trypan red, and this was confirmed by Franke (1905). In the case of his strain of *T. brucei*, Ehrlich (1907) declared that the period of immunity may sink down to 10 days. Schilling (1909) and Schilling and Jaffé (1909), working with *T. equinum* and *T. brucei*, and with trypan red, atoxyl and arseno-phenylglycine, appear to have found that the immunity-period in mice after a single treatment, while varying considerably, both in individual instances and according to the particular combination tested, was, in general, no longer, and frequently much shorter, than the periods just mentioned. Browning (1908) also reported a duration of immunity of not more than 10 days following the treatment of mice for infections with a strain of *T. brucei*. Neumann (1911), working with *T. brucei* and arsacetin, showed the immunity in 3 of 5 mice to last no longer than 27 days, although, indeed, in the remaining 2 cases the period lasted respectively for 56 and for at least 70 days. Terry (1911), in immunity studies on mice treated by various drugs for infections with 3 strains of *T. evansi* and with strains of *T. equinum*, *T. equiperdum* and *T. brucei*, also found the immunity to be transitory : ' Sometimes . . . it disappeared completely in eight to eleven days, and in animals tested but once it was unusual to find much resistance twenty days after treatment.' Von Jancsó and von Jancsó (1934) say that immunity to their strain of *T. brucei* lasts ' for some weeks ' after treatment. It is evident, therefore, in comparing our own findings with these various reports, that, whether the explanation lies in differences in the strains employed or in differences of technique, the immunity acquired by mice after treatment of a trypanosome infection may not be as transient as appears to have been generally credited hitherto.

III. DEMONSTRATION OF TRYPANOLYSIS *IN VITRO* BY MOUSE IMMUNE SERUM, IN THE PRESENCE AND IN THE ABSENCE OF HAEMOLYTIC COMPLEMENT

(*a*) *Trypanolysis in the presence of complement*

The following is an account of an experiment in which trypanolysis by mouse immune serum was demonstrated *in vitro* :—

EXPERIMENT.—Fourteen mice were infected with *T. rhodesiense*, and were treated by 0·08 mgm. halarsol two days later, when there were about 1 to 10 parasites per microscopic field (oc. 4, obj. 1/6th). After a further five days the mice were killed, and their sera pooled. The sera of 14 normal mice were also pooled, to serve as a control. Dilutions were then made in a medium consisting of equal volumes of fresh unheated

guinea-pig serum and Ringer-glucose solution. These dilutions were distributed among small tubes, in volumes of 0·45 c.cm. Each tube was then sown with 1/40th c.cm. of a suspension of washed homologous trypanosomes in a mixture of Ringer-glucose solution—3 parts—and guinea-pig serum—1 part. The strength of the suspension was such that the tubes when sown each contained about 1,000 trypanosomes per c.mm. (See Yorke, Adams and Murgatroyd, 1929.) The tubes were then kept at 37° C., and examined at various intervals during the next 24 hours.

TABLE II

Showing trypanolytic effect *in vitro* of mouse immune serum (*a*) undiluted, and (*b*) diluted in unheated guinea-pig medium*

Mouse serum tested	Tube	Dilution of mouse serum in unheated guinea-pig medium	No. of living trypanosomes per 256 squares of the haemocytometer scale							
			Start	½ hour	2 hours	4 hours	6 hours	10 hours	14 hours	18 hours	24 hours
Immune	1	Undiluted	70	78	23	12	11	1	0	...	...
	2	1 : 2		5	2	0	...	...	...	...	...
	3	1 : 4		0	...	...	...	...	...	...	...
	4	1 : 8		0	...	...	...	...	...	...	...
	5	1 : 16		0	...	...	...	...	...	...	...
	6	1 : 32		0	...	...	...	...	...	...	...
	7	1 : 64		0	...	...	...	...	...	...	...
	8	1 : 128		0	...	...	...	...	...	...	...
	9	1 : 256		27	25	22	31	33	32	31	37
	10	1 : 512		76	...	...	...	...	...	...	66
	11	1 : 1,024		...	...	...	...	...	...	...	72
	12	1 : 2,048		...	...	...	...	...	...	...	72
Normal	13	Undiluted		72	68	...	...	76	...	28	24
	14	1 : 2		...	80	88	...	64	...	72	68
	15	1 : 4		64	68	62	60	86	90	84	76
	16	1 : 8		...	...	...	...	...	...	...	58

* Medium consists of equal parts guinea-pig serum and Ringer-glucose solution.

The results given in Table II show that, in the presence of complement (Tubes 2–12), trypanolysis by the immune serum in dilutions up to 1 : 128 was very rapidly effective, the organisms being destroyed within half an hour. In Tube 2, however, there was some delay in the lytic action ; we have frequently

observed this form of ' zone '* effect, in which the highest concentrations of mouse immune serum in a medium containing guinea-pig or rabbit serum does not exhibit complete trypanolysis for some hours, while much lower concentrations are effective in a considerably shorter space of time. We will return later to this point and offer an explanation for its occurrence. It should be noted that the titre was not increased when the observation period was prolonged from half an hour to 24 hours (see Tubes 8 and 9). In the case of Tube 1, containing undiluted immune serum, there was clearly, in spite of the absence of guinea-pig complement, a very definite, although slow-acting, trypanocidal effect. While the trypanosomes in this tube had not been reduced in number within half an hour, they had become sluggish and somewhat distorted ; gradually their numbers began to diminish, until in 10–14 hours they had all disappeared.

Turning now to the control series, it is seen (Tubes 14–16) that mixtures of normal mouse and guinea-pig serum serve as suitable supporting media for the trypanosomes. We have never encountered a trypanocidal process of any account in mixtures of normal mouse and guinea-pig (or rabbit) serum ; and it may be mentioned that, although in this experiment the normal mouse serum has only been diluted up to 8 times, in other experiments we have frequently carried the dilutions to the same level as in the experimental series. It must be noted, though, that there was a definite diminution in the number of trypanosomes in the tube containing undiluted normal mouse serum (Tube 13) ; after 24 hours the number per 256 squares of the haemocytometer scale had fallen from about 70 to 24, and those surviving were slowly motile and abnormal in form. We have generally found that pure mouse serum is not an entirely satisfactory supporting medium for trypanosomes ; in some of our experiments the parasites in this environment were all destroyed at the end of 24 hours, although occasionally the full number survived apparently unharmed. In the case of undiluted immune serum, however, the result has been constant ; in half an hour there was invariably a disturbance of motility and form, and the trypanosomes always disappeared entirely before there was any effect in normal serum. In the experiment described above it was unnecessary to provide controls to exclude trypanolysis due to possible unexcreted or unmetabolized drug, since other experiments have shown that this complication certainly does not arise on the fifth day after even the heaviest dose of halarsol.

The next step was to show the effects of heat upon the immune serum (Table III). Two portions of each serum used in the previous example were heated respectively to 56° C. and 64° C. for half an hour, and the experiment was repeated. The results show that heating to 56° C. does not affect the outcome (cf. Table III, Tubes 17–27, with Table II). Another experiment showed

* We are using this term here with strict reserve, since, although the effect recorded above is somewhat similar, we do not intend to imply that it comes into the same category of reactions, in regard to its explanation, as the well-known Neisser-Wechsberg phenomenon in bacteriology, to which the terms ' zone ' and ' prozone ' phenomena have been applied.

TABLE III

Showing trypanolytic effect *in vitro* of mouse immune serum, previously heated for half an hour to 56° C. and to 64° C., (*a*) undiluted, and (*b*) diluted in unheated guinea-pig medium*

Mouse serum tested	Mouse serum previously heated to	Tube	Dilution of mouse serum in unheated guinea-pig medium	No. of trypanosomes per 256 squares of the haemocytometer scale							
				Start	½ hour	2 hours	4 hours	6 hours	10 hours	14 hours	18 hours	24 hours
Immune	56° C.	17	Undiluted	70	62	50	62	37	7	1	0	...
		18	1 : 2		0	...	...	...	...	...	...	...
		19	1 : 4		0	...	...	...	...	...	...	...
		20	1 : 8		0	...	...	...	...	...	...	...
		21	1 : 16		0	...	...	...	...	...	...	...
		22	1 : 32		0	...	...	...	...	...	...	...
		23	1 : 64		0	...	...	...	...	...	...	...
		24	1 : 128		0	...	...	...	...	...	...	...
		25	1 : 256		44	52	54	56	66	50	62	68
		26	1 : 512		...	...	...	...	...	...	...	88
		27	1 : 1,024		...	...	...	...	...	...	...	84
	64° C.	28	Undiluted		88	35	34	22	2	0	...	...
		29	1 : 2		0	...	...	...	...	...	...	...
		30	1 : 4		0	...	...	...	...	...	...	...
		31	1 : 8		31	14	18	17	4	6	7	4
		32	1 : 16		70	80	84	80	64	52	60	52
		33	1 : 32		...	...	...	...	...	...	...	80
		34	1 : 64		...	...	...	...	...	...	...	74
Normal	56° C.	35	Undiluted		84	92	...	...	60	...	41	31
		36	1 : 2		...	56	76	...	56	...	60	52
		37	1 : 4		56	62	78	66	68	62	68	58
		38	1 : 8		...	...	...	...	...	...	...	80
	64° C.	39	Undiluted		56	51	...	...	52	...	23	14
		40	1 : 2		...	64	58	...	54	...	...	56
		41	1 : 4		62	54	86	92	94	86	88	84
		42	1 : 8		...	...	...	...	...	...	...	80

* Medium consists of equal parts guinea-pig serum and Ringer-glucose solution.

that this result also holds for heating to 60° C. A temperature of 64° C., however, has a very definite effect, the titre being reduced in the experiment of Table III from 128 to 4, (see Tubes 28–34). Incidentally it is interesting to note that the falling off in number of trypanosomes observed in normal unheated mouse serum (Table II, Tube 13) is equally to be observed if that serum has previously been heated (Table III, Tubes 35 and 39).

(*b*) *Trypanolysis in the absence of complement*

With the object of gaining further information as to whether complement is really essential for trypanolysis, an experiment (Table IV) was next performed in which our pooled mouse serum was diluted in a medium of equal parts of Ringer-glucose solution and guinea-pig serum of which the haemolytic complement had been destroyed by previously heating to 56° C. for half an hour. In this way we tested the mouse serum both unheated and heated to 56° C. for half an hour. It should be stated that, in the experiments of this section we were careful to heat to 64° C. for half an hour the mixture of guinea-pig serum and Ringer-glucose solution in which the trypanosomes had been washed and suspended prior to sowing the experimental tubes. Considering first the findings with the unheated immune serum, in the experiment of Table IV (Tubes 43–50), it may be seen that a trypanolytic action did take place, but the titre was somewhat reduced, being 32 as compared with 128 in the presence of unheated guinea-pig serum (Tables II and III). Furthermore, trypanolysis took somewhat longer to become fully effective than had been the case in the presence of complement ; instead of the action being complete in half an hour, the process took about 2 hours. If one accepts the statement of Ritz (1911) that mouse serum lacks haemolytic complement, then we have here a clear exhibition of *in vitro* trypanolysis by immune serum in the absence of haemolytic complement. However, it would be unwise to exclude the possibility of there being at least traces of complement in mouse serum, so that the evidence supplied by the dilutions of heated immune serum (Tubes 51–58) is, perhaps, a more satisfactory basis for the statement that trypanolysis takes place in the complete absence of haemolytic complement. In this case it is seen that there was a titre of only about 16, and the action was slow, so that the trypanosomes were not destroyed until 14–18 hours after sowing.

It is to be noted that, while heating the immune serum to 56° C. for half an hour had resulted in no appreciable difference in the effects observed in the presence of complement (cf. Table II, Tubes 2–12, with Table III, Tubes 18–27), yet, in the absence of complement (Table IV, cf. Tubes 43–50 with Tubes 51–58), there was a definite difference, particularly in regard to the speed of action, and also, perhaps, in regard to the final titre attained. We have not yet determined whether this difference is to be accounted for by the fact that there may indeed be traces of complement in unheated mouse serum, or whether

TABLE IV

Showing trypanolytic effect *in vitro* of mouse immune serum (*a*) unheated and (*b*) previously heated to 56° C. for half an hour, diluted in guinea-pig medium* previously heated to 56° C. for half an hour

Mouse serum tested	Whether mouse serum previously heated or not	Tube	Dilution of mouse serum in guinea-pig medium previously heated to 56° C.	No. of trypanosomes per 256 squares of the haemocytometer scale							
				Start	½ hour	2 hours	4 hours	6 hours	10 hours	14 hours	18 hours	24 hours
Immune	Unheated	43	1 : 2	70	60	1	0	...	...	...	...	...
		44	1 : 4		42	0	...	...	...	...	...	...
		45	1 : 8		40	0	...	...	...	...	...	...
		46	1 : 16		47	1	0	...	...	...	...	...
		47	1 : 32		76	0	...	...	...	...	...	...
		48	1 : 64		68	52	62	70	54	66	70	56
		49	1 : 128		...	...	...	...	...	...	...	74
		50	1 : 256		...	...	...	...	...	...	...	76
	56° C.	51	1 : 2		84	68	52	23	11	6	0	...
		52	1 : 4		64	38	42	41	10	9	0	...
		53	1 : 8		74	80	43	50	8	3	0	...
		54	1 : 16		90	60	33	...	19	11	2	0
		55	1 : 32		68	...	...	...	66	37	39	42
		56	1 : 64		...	...	...	...	70	...	49	43
		57	1 : 128		...	...	...	...	...	...	...	68
		58	1 : 256		...	...	...	...	...	...	...	76
Normal	Unheated	59	1 : 2		...	70	...	...	78	...	66	60
		60	1 : 4		...	92	90	...	80	...	82	90
		61	1 : 8		78	82	86	86	90	92	70	74
	56° C.	62	1 : 2		...	76	...	...	92	...	74	68
		63	1 : 4		...	72	...	...	88	...	96	92
		64	1 : 8		84	90	94	78	96	92	98	86

* Medium consists of equal parts guinea-pig serum and Ringer-glucose solution.

the explanation lies rather in an effect of heat, at 56° C., directly upon the immune-body itself—an effect which does not become evident in the presence of an excess of haemolytic complement.

In the experiments described above there is, then, adequate evidence of the occurrence of trypanolysis *in vitro* in the complete absence of haemolytic complement, and this conclusion is still further borne out by the results figured in Table V. These findings refer to an experiment in which

TABLE V

Showing trypanolytic effect *in vitro* of mouse immune serum, previously heated to 64° C. for half an hour, diluted in guinea-pig medium* similarly heated

Mouse serum tested	Tube	Dilution of mouse serum in guinea-pig medium, both previously heated to 64° C.	No. of trypanosomes per 256 squares of the haemocytometer scale							
			Start	½ hour	2 hours	4 hours	6 hours	10 hours	14 hours	18 hours	24 hours
Immune	65	1 : 2	70	68	62	32	12	4	5	2	0
	66	1 : 4		82	68	36	...	4	...	2	0
	67	1 : 8		...	...	...	...	32	...	6	1
	68	1 : 16		...	...	...	...	...	...	70	66
	69	1 : 32		...	...	...	...	...	...	...	74
	70	1 : 64		...	...	...	...	...	...	...	66
Normal	71	1 : 2		...	88	...	...	78	...	...	60
	72	1 : 4		...	72	58	...	78	...	...	82
	73	1 : 8		70	62	76	68	90	80	88	94

* Medium consists of equal parts guinea-pig serum and Ringer-glucose solution.

we heated to 64° C. for half an hour another portion of the pooled mouse serum, as well as the guinea-pig serum used for the diluting medium. It is hardly to be believed that there can be any further traces of haemolytic complement in serum which has been subjected to such a degree of heat, yet here, even though the immune-body has been seriously assaulted (see Table III, Tubes 28-34), we still have definite evidence of slow-acting trypanolysis. The titre was about 8, and in dilutions up to this point a decline in the number of trypanosomes was already to be noted at the fourth hour, although it took from 18 to 24 hours for all to be destroyed.

IV. CERTAIN ASPECTS OF THE RÔLE OF COMPLEMENT IN TRYPANOLYSIS *IN VITRO*

(*a*) *Delayed trypanolysis sometimes observed in higher concentrations of mouse immune serum, in the presence of complement*

The experiments detailed in the preceding section have shown, in brief, that trypanolysis by mouse immune serum is very rapidly effective in the presence of added haemolytic complement, but that it occurs also, although operating in much slower measure and not in as high a dilution, under various conditions where there is no haemolytic complement present. Attention has already been drawn to the fact that in the presence of complement what may be described as a form of ' zone ' effect often takes place ; in this, complete trypanolysis does not occur for some hours in a higher concentration of mouse immune serum, while it is fully effective in a much shorter space of time in lower concentrations. This phenomenon was encountered particularly when the complement-content of the diluting medium was low, as when rabbit or stored guinea-pig serum was used. Now it is evident that, with the technique described, the tubes containing the highest concentrations of immune serum necessarily include a lower proportion of the diluent serum—and hence of complement—than the remaining tubes. It might be suspected, therefore, that, since the presence of complement is evidently necessary for rapid trypanolysis, then, especially when the diluting medium is poor in complement, the effect of a lower proportion of that component being present in the first tubes of the series might be sufficient to cause trypanolysis to be delayed in those as compared with succeeding tubes.

That the above explanation does not, however, account for the effect we are considering is made clear by the results of the following experiment :—

EXPERIMENT :—Two parallel series of dilutions of a sample of mouse immune serum were made as follows (see Table VI) :—

(*a*) In a medium of equal parts of fresh rabbit serum and Ringer-glucose solution, by our customary technique, so that the relative amounts of rabbit serum increased from 25 per cent. in Tube 74 to nearly 50 per cent. in Tube 79.

(*b*) In Ringer-glucose solution, a constant amount of rabbit serum being subsequently added, so that the latter should be present in the proportion of 25 per cent. in each tube (Nos. 81–87).

The experiment was so arranged that the final dilutions of immune serum in each series ranged from 1 : 2 to 1 : 64, the only difference being, therefore, that in series (*a*) the complement was present in increasing amounts in successive tubes, as in our other experiments, while in series (*b*) it was present in constant amount. The tubes were then sown, in the usual way, with trypanosomes suspended in a mixture of rabbit serum and Ringer-glucose solution.

It will be seen that the form of ' zone ' effect with which we are concerned appeared equally in both series, so that its occurrence is not to be accounted for by the fact that, with our customary diluting technique, there is relatively less complement in the first tubes of the series than in the remainder.

We believe that an adequate explanation for the phenomenon under consideration is to be found in an anti-complementary action of mouse serum.

TABLE VI

Showing trypanolysis *in vitro* by mouse immune serum in dilutions with Ringer-glucose solution in the presence of (*a*) increasing amounts of fresh rabbit serum in successive tubes, and (*b*) constant amounts of fresh rabbit serum

	Tube	Dilution of mouse immune serum	Percentage of rabbit serum present	No. of trypanosomes per 256 squares of the haemocytometer scale		
				Start	1½ hours	24 hours
Series (*a*)	74	1 : 2	25 (approx.)	50	17	0
	75	1 : 4	38 ,,		13	0
	76	1 : 8	44 ,,		4	0
	77	1 : 16	47 ,,		0	...
	78	1 : 32	48 ,,		0	...
	79	1 : 64	49 ,,		0	...
	80	—	50		46	52
Series (*b*)	81	1 : 2	25		16	0
	82	1 : 4			15	0
	83	1 : 8			3	0
	84	1 : 16			0	...
	85	1 : 32			0	...
	86	1 : 64			0	...
	87	—			54	48

TABLE VII

Showing anti-complementary action of mouse serum in a haemolytic system

Tube	A 0·45 c.cm.	+	B 0·225 c.cm.	+	C 0·225 c.cm.	Result
1	Undiluted mouse serum		Rabbit serum, 1 part : saline, 4 parts		Sensitized sheep red cells	No haemolysis
2	Mouse serum diluted in saline 1 : 2					,, ,,
3	1 : 4					,, ,,
4	1 : 8					Partial haemolysis
5	1 : 16					Haemolysis
6	1 : 32					,,
7	1 : 64					,,
8	Saline					Haemolysis

A and B were mixed and left standing for half an hour at 37° C., before adding C. Result was read after a further half hour at 37° C.

This action is illustrated by the following experiment (see Table VII) :—

EXPERIMENT.—The serum of two normal mice was pooled, and 0·45 c.cm. of dilutions of this serum in saline were then added to tubes containing 0·225 c.cm. of a 1 in 5 mixture of rabbit serum and saline. The tubes were shaken and allowed to stand at 37° C. for half an hour ; then 0·225 c.cm. sensitized sheep red corpuscles was added to each tube and, after further shaking and standing at 37° C. for another half hour, readings were made.

It will be seen from the Table that the presence of the higher concentrations of mouse serum in the haemolytic system served to inhibit haemolysis by the complement of the rabbit serum. It would seem reasonable to suppose that it is this anti-complementary property in mouse serum which is responsible for the delayed trypanolysis which we have described above as sometimes occurring, in the presence of complement, in tubes containing higher concentrations of mouse immune serum.

(*b*) *Determination of titre of complement required for rapid* in vitro *trypanolysis*

Our next step was to arrive at an estimate of the amount of complement required for the more rapid type of *in vitro* trypanolysis. With this end in view, the following experiment was performed :—

EXPERIMENT.—A large number of trypanosomes (*T. rhodesiense*) was obtained from a heavily infected mouse, washed and then brought into contact, at 0° C. for 1 hour, with about 0·5 c.cm. of the same stock of immune serum as was used for the experiments of Tables II–V ; the serum had previously been heated to 56° C. for half an hour. The trypanosomes thus sensitized were then washed three times in a mixture of 1 part Ringer-glucose solution and 1 part guinea-pig serum previously heated to 64° C. ; they were finally suspended in the latter medium, in a concentration suitable for sowing the tubes of Table VIII. Tubes 9-18 and 21-23 contained dilutions of complement (fresh unheated guinea-pig serum) in a medium of equal parts Ringer-glucose solution and guinea-pig serum previously heated to 64° C.; tubes 19 and 20 contained no complement. As a control, exactly similar procedures were adopted with trypanosomes placed in contact, at 0° C., with normal, instead of immune, mouse serum.

The results figured in Table VIII show that a concentration of no more than 1 : 16–1 : 32 of complement (unheated guinea-pig serum) is sufficient for trypanolysis to take place within half an hour, at least under the conditions of this experiment. It is to be noted that, while sensitized trypanosomes are destroyed within half an hour in the presence of complement, no such effect occurs, during a longer observation period, in its absence (Tubes 19 and 20). A slow lytic process in the latter circumstance might perhaps not have been unexpected in view of the evidence we have provided that delayed trypanolysis may occur in the entire absence of complement (see Tables III, IV and V.).

Before leaving this experiment it should be mentioned that we tested the trypanolytic power of the absorbed immune serum, after removing the trypanosomes that had been sensitized by its influence. The titre proved still to be 128, which was exactly the same as before absorption (see Table II). The amount of immune-body removed by the trypanosomes was, therefore, negligible, and

the latter had obviously been sensitized in the presence of a great excess of immune-body. Incidentally, Taliaferro (1932), referring to *T. lewisi*, states that at least 1,000,000 parasites are needed to absorb 1 c.mm. of immune serum completely. A rough estimate is that, for the sensitization of our trypanosomes,

TABLE VIII

Showing (*a*) the titre of complement required for rapid lysis of sensitized trypanosomes, and (*b*) failure of lysis of sensitized trypanosomes, during a period of 24 hours, in the absence of complement

Trypanosomes used	Tube	Trypanosomes sown in :—		No. of trypanosomes per 256 squares of the haemocytometer scale		
				Start	½ hour	24 hours
Sensitized	9	Dilutions of unheated guinea-pig serum in guinea-pig medium* previously heated to 64° C. for half an hour	Undiluted	62	0	...
	10		1 : 2		0	...
	11		1 : 4		0	...
	12		1 : 8		0	...
	13		1 : 16		0	...
	14		1 : 32		9	4
	15		1 : 64		45	35
	16		1 : 128		72	68
	17		1 : 256		70	52
	18		1 : 512		54	44
	19	Guinea-pig serum previously heated to 64° C. for half an hour			68	48
	20	Guinea-pig serum previously heated to 56° C. for half an hour			60	50
Unsensitized	21	Dilutions of unheated guinea-pig serum, in guinea-pig medium previously heated to 64° C. for half an hour	Undiluted	74	74	62
	22		1 : 2		64	56
	23		1 : 4		78	70

* Medium consists of equal parts guinea-pig serum and Ringer-glucose solution.

we must have employed only about 17–18,000 organisms per c.mm. of immune serum. This point is being made in connection with the present experiment, since the titre of complement for rapid trypanolysis may well be lower in the case of lightly sensitized trypanosomes than in the case of organisms sensitized in the presence of an excess of immune-body.

V. STRAIN-SPECIFICITY OF TRYPANOLYSIS *IN VITRO* BY MOUSE IMMUNE SERUM

It has been pointed out in the Introduction that we have readily elicited trypanolysis *in vitro* by the serum of mice immune respectively to each of the five separate strains of trypanosome mentioned. We have made no cross-tests between any of these strains and the five corresponding immune sera, but a high degree of specificity in the trypanolytic process *in vitro* is indicated by the results of a cross-test employing our *T. rhodesiense* and an off-shoot of the same strain, derived from a mouse which had relapsed after treatment (see Table IX).

TABLE IX

Showing specificity of trypanolysis *in vitro* by sera from mice immune respectively to a strain of *T. rhodesiense* and to a relapse off-shoot from the same strain

Immune serum tested	Trypanosomes tested	Tube	Dilution of immune serum in unheated guinea-pig medium*	No. of trypanosomes per 256 squares of the haemocytometer scale	
				Start	½ hour
Parent	Parent	24	1 : 8	100	0
		25	1 : 16		0
		26	1 : 32		0
		27	1 : 64		70
		28	1 : 128		108
		29	1 : 256		98
	Relapse	30	1 : 2		104
		31	1 : 4		96
		32	1 : 8		102
Relapse	Parent	33	1 : 2		104
		34	1 : 4		100
		35	1 : 8		96
	Relapse	36	1 : 256		0
		37	1 : 512		0
		38	1 : 1,024		0
		39	1 : 2,048		49
		40	1 : 4,096		94
		41	1 : 8,192		102

*Medium consists of equal parts guinea-pig serum and Ringer-glucose solution.

The finding is, of course, to be expected, in view of the fact shown by Ehrlich (1909), and amply confirmed by many others, that a passage strain in mice and its relapse off-shoot are easily distinguishable by cross-immunity tests ; indeed, it has frequently been shown that greater immunological differences may exist between a strain and its relapse variants than between trypanosomes which are generally recognized to be of different, but related, species (see review by Taliaferro, 1930).

The variant used for the experiment of Table IX arose from a mouse which had relapsed 10 days after treatment of an infection with *T. rhodesiense*. The parasites had subsequently been maintained in mice, and, after 45 passages, about $4\frac{1}{2}$ months since isolation, two infections were treated by 0·32 mgm. halarsol ; five days later the mice were bled, and their sera pooled, to provide the relapse strain immune serum. The parent strain immune serum was obtained from a single mouse, treated 20 days previously by 0·64 mgm. halarsol, for an infection with the appropriate parasites.

As may be seen from the Table, the action *in vitro* of each of the two sera was strictly confined to the corresponding trypanosomes. The parent strain immune serum proved to have a titre of 32 against the homologous parasites, and exercised no effect against the relapse trypanosomes, while the relapse strain immune serum was active against its corresponding strain in a titre of 1,024, and was entirely without effect against the parent trypanosomes. The wide discrepancy between the two titres obtained is not to be explained by any quantitative difference in the antigenic properties of the two strains, but by the different time periods in each case (5 days and 20 days respectively) between treatment of the infection and testing of the serum. This matter is considered in greater detail in the next section of this contribution.

VI. INFLUENCE UPON THE TRYPANOLYTIC TITRE OF:— (*a*) TIME SINCE TREATMENT; (*b*) DEGREE OF INFECTION AT TIME OF TREATMENT; (*c*) SIZE OF DOSE

In Table X are recorded the results of examination for trypanolysis *in vitro*, in the presence of complement, of a large number of mouse immune sera. The titre of each serum tested is placed, on the Table, in relation to (*a*) the time since treatment, (*b*) the degree of infection at the time of treatment, and (*c*) the dose of halarsol given. The majority of the sera were from single mice, and the remainder were pooled from two or more cases ; however, each sample of pooled serum came from mice which were equivalent to one another in regard to all three of the above-named factors. The results of examination of these pooled sera are distinguished by an asterisk, on the Table, from results referring to sera of individual mice. The majority of the results recorded relate to examinations of *T. rhodesiense* immune serum, but the Table also includes the values obtained for immune sera for each of our other four strains (as well as the *T. rhodesiense* relapse off-shoot referred to in the preceding section). These

latter figures may be identified by suitable symbols, which accompany them and which are explained at the foot of the Table ; it will be seen that they all fit perfectly into the various curves for *T. rhodesiense* immune serum. Before passing on to a fuller consideration of the results, attention may be drawn to the fact that some of the figures (see Series 3 and 8) refer to titres obtained only 2 days after treatment by as much as 0·64 mgm. of halarsol. It might well be expected that, in these cases, *in vitro* tests may be complicated by unexcreted and undestroyed drug in the serum tested ; however, we have ascertained that, although the serum of uninfected mice treated 2 days previously by 0·64 mgm. halarsol does indeed destroy trypanosomes in a dilution of 4 or 8, this action does not take full effect until some 24 hours. Within half an hour there is no appreciable action whatever ; the possible complication mentioned above does not arise, therefore, since all our experimental tubes were read half an hour after sowing. We have already seen that, in the presence of complement, the same titre for trypanolysin is obtained at the end of half an hour as after a period of 24 hours.

Examination of the findings collected on Table X shows that, in regard to the degree of infection at the time of treatment, we have arranged the cases into three main groups, of which one comprises infections in which there were many thousands of times as many parasites (Series 6 to 8) as in the group of infections of lowest degree (Series 1 to 3). The dosages of halarsol ranged from the minimum effective amount—0·02 mgm. per 20 gm. mouse—to a dose 32 times as great—0·64 mgm., which is close upon the lethal dose for mice. It is to be noted that all the mice were treated on the second day of infection, the size of the inoculum having been adjusted in each case according to the degree of infection required two days later. Considered as a whole, the results show that, while there is, as might be expected, a definite degree of individual variation between different results in the same grouping, the general trend is for the titre to rise steeply during the first few days subsequent to treatment, reaching a high level, which may be as much as 1,024 or even 2,048, between about the fourth and eleventh days. There follows a gradual decline, occupying, apparently, about the next 3 or 4 weeks, till a low range of between 2 and 32 is reached, and this is maintained for many months, during which time, however, the titre does sometimes sink below 2. The observations were continued at intervals for as long as 8 months (252 days), and it is seen that, at the expiration of that period, trypanolysins were still demonstrable *in vitro*, though in low concentration, in two of the three specimens examined. When we look in closer detail for the influence of the degree of infection at the time of treatment, and of the dose of drug given, we see that, irrespective of the size of the dose, there is a definite lag in the curve of rising titre in serum from those mice in which the infections were of very low grade at the time of treatment (cf. Series 1 and 3 with 4 and 8). The only other difference that is at all noticeable is that the figures relating to the very low grade infections treated by 0·64 mgm.

TABLE X.—Showing the influence of (*a*) time since treatment, (*b*) degree of infection at

No. of parasites per field at time of treatment	Dose of halarsol	No. of									
		2	3	4	5	6	7	8	9	10	11
$\frac{1}{800}$ to $\frac{1}{20}$	0·02 mgm. [Series 1]	<2			256					64	
		<2			512					256	
		<2			1024					512	
		<2			2048					512	
		<2									
		4									
	0·08 mgm.										
	0·64 mgm. [Series 3]	<2			32					8	
		<2			32					16	
		<2			32					64	
		<2			256					128	
		<2			512						
		<2									
1 to 20	0·08 mgm. [Series 4]	32	128	256	128*	128		256*		256*	256* b.f.
					1024	512*					
	0·16 mgm. [Series 5]				128*	256*	256* b.p.	64*		256*	
					512*	256* ep.		512*			
								512 en.			
Swarming	0·04 mgm. [Series 6]					256*					256
											256
											256*
	0·32 mgm.				1024* r.r.						
	0·64 mgm. [Series 8]	8			64				512	64	
		8			512					128	
		32			512					128	
		32			1024					256	
		64								256	
		128									

* Pooled serum examined ; all other figures represent titres of serum from single mice. ep. = *T. equiperdum* ;

time of treatment, and (*c*) size of dose, upon the trypanolytic titre of mouse immune serum

Trypanolytic titre															
days since drug treatment															
12	13	14	15	16	18	19	20	25	30	121	134	143	185	240	252
			16				16	16							
			32				32	64							
			64				64	64							
			128				128	64							
										<2 b.f.					
			16				4	<2							
			32				16	8							
			32				16	128							
			64				64								
128*	64*		64*	16	64*				8	8 b.f.	32	16 b.f.	2	<2	<2 b.f.
	128			16											
				32											
						32*			16						4 b.f.
															8 b.f.
		128													
			16				4	8							
			128				32	32							
			128				32	32							
			128				32	64							

en. = *T. equinum* ; b.p. = *T. brucei*, parent strain ; b.f. = *T. brucei*, fly strain ; r.r. = *T. rhodesiense* relapse off-shoot ; all remaining results refer to *T. rhodesiense* immune sera.

halarsol (Series 3) perhaps appear not to reach quite as high a level as the figures for the other infections. However, this difference is not a great one, and, in the main, one is justified in summarizing the situation by the statement that the curve is not influenced to any marked extent either by the degree of infection at the time of treatment or by the dose of drug given—although there is a slight delay in the appearance of trypanolysins in the case of lightly infected mice treated either by a small or a large dose.

VII. RETENTION OF TRYPANOLYTIC TITRE BY IMMUNE SERUM STORED AT 0° C.

It is stated by Schilling, Schreck, Neumann, and Kunert (1936) that immune serum must always be fresh, for the demonstration of antibodies towards trypanosomes, and they give as an example that the serum of a calf almost entirely lost its activity on being kept for 9 days in the ice-box. In view of this statement it is of interest to set forth Table XI, in which we show that mouse immune sera of various strengths retained their *in vitro* trypanolytic potency quite unimpaired, when stored in the ice-box for periods up to 6 weeks.

TABLE XI

Showing retention of trypanolytic titre by mouse immune serum stored at 0° C.

No. of specimen	Time during which immune serum was stored at 0° C.	Titre on day serum was taken	Titre after storing at 0° C.
1	41 days	128–256	128
2	35 ,,	16	16
3	28 ,,	256	256
4	10 ,,	256–512	512
5	10 ,,	256	256
6	5 ,,	64	64
7	5 ,,	512	512

VIII. SUMMARY AND CONCLUSIONS

1. It is well known that, following suitable drug treatment of mice infected with one of the rapidly fatal strains of trypanosome, there is an immunity, or relative immunity, to reinfection with the same strain. Since the work of Ehrlich and Shiga (1904) it has been repeatedly reaffirmed by a considerable number of workers, to some of whom we have referred in the text, that this immunity is of a very transient nature, generally being lost in the course of a few weeks. However, in our experience, immunity to reinfection is generally retained for at least as long as 8 months after treatment.

2. Although trypanolysis *in vitro* by immune serum of larger animals has been established for more than 30 years, very scant attention has hitherto been paid to the demonstration of this phenomenon by mouse immune serum. Several writers declare that trypanolysis *in vitro* by mouse immune serum does not occur at all, or does so only occasionally and in very slight degree—and this failure has been ascribed to the lack of complement in mouse serum. We have found that trypanolysis by mouse immune serum may readily be demonstrated *in vitro* at 37° C., not only in the presence of added haemolytic complement, but also in the entire absence of that factor. In the former case the trypanocidal action is complete within half an hour, and the titre is relatively high ; in the latter case the action is much more protracted, and the titre is lower. In some instances, however, in the presence of added complement, trypanolysis is unduly delayed in higher concentrations of mouse immune serum, while being rapidly effective in lower concentrations. We give experimental support for the belief that this anomaly is due to an anti-complementary action of mouse serum.

3. Trypanosomes have been sensitized, through contact with mouse immune serum at 0° C. for 1 hour. By washing and then distributing these parasites among tubes containing dilutions of haemolytic complement (fresh guinea-pig serum), it has been found that, under the conditions of our experiment, a concentration of 1 : 16 – 1 : 32 of complement is sufficient for trypanolysis to take place within half an hour at 37° C. In the absence of complement, however, trypanosomes sensitized in the above manner do not undergo lysis during a period of 24 hours.

4. Figures are given showing the influence upon the trypanolytic titre of mouse immune serum (in the presence of complement) of the following factors :—(*a*) time since treatment, (*b*) degree of infection, and (*c*) size of dose. The general trend is for the titre to rise steeply during the first few days, reaching a high level, which may be as much as 1,024, or even 2,048, between about the fourth and eleventh days. There follows a gradual decline during the next 3 or 4 weeks, till a low range of between 2 and 32 is reached, and this is maintained, with a few defections to below 2, for at least as long after treatment as 8 months. The curve is apparently not markedly influenced either by the degree of infection at the time of treatment, or by the dose of drug given, although there is indeed a slight delay in the appearance of trypanolysins in the case of lightly infected mice treated either by a small or a large dose.

5. Trypanolysis *in vitro* of the homologous parasites has been observed by sera from mice immune respectively to each of five strains (comprising four separate species) of pathogenic trypanosome. While we have made no cross-tests between any of these sera and the five corresponding strains, a high degree of specificity has been shown between mouse immune serum for our *T. rhodesiense*, on the one hand, and immune serum for a relapse off-shoot of the same strain, on the other. The trypanolytic titres attained, at various times after treatment, by immune sera for each of the strains (including the relapse off-shoot

just mentioned) upon which fewer observations have been made, all fit perfectly into the curve of titre for immune serum in respect of the strain of *T. rhodesiense* upon which the greater part of the present work has been done.

6. The trypanolytic titre of mouse immune serum, as tested in the presence of complement, is unaffected by heating to 60° C. for half an hour, but is materially reduced by a temperature of 64° C. for half an hour. The serum may be stored at 0° C. for at least 6 weeks, without any loss in its potency.

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EXPERIMENTAL TRANSMISSION OF *TRYPANOSOMA GAMBIENSE* BY *GLOSSINA MORSITANS* THROUGH MONKEYS

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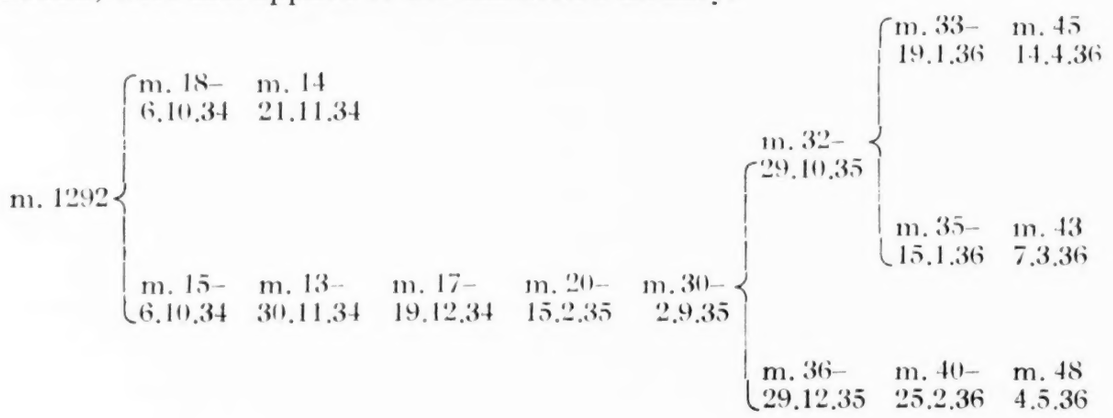
INTRODUCTION

In the paper in which they described and named *Trypanosoma rhodesiense*, Stephens and Fantham (1910) said that the trypanosome might possibly be a variety or race of *T. gambiense* due to some change of environment, and that the peculiar morphology of the new trypanosome might be the result of its having been conveyed by a species of *Glossina* other than *Glossina palpalis*. They added that we had no knowledge of any other trypanosome undergoing similar changes owing to such a cause, and that they considered that they were dealing with a new species of trypanosome. The only experimental evidence in support of the idea that *T. rhodesiense* has been produced by the passage of *T. gambiense* through a species of *Glossina* other than *G. palpalis* was given by Kleine (1928), who wrote : ' Moreover, W. Fischer (1913), on Lake Tanganyika, reported to me on the strikingly rapid death of monkeys which he had infected there by means of *G. morsitans* with *T. gambiense*. I regret that we did not pay more attention to these experiments.' Kleine and Fischer (1913), in parallel experiments with 13 strains of *T. gambiense*, succeeded in transmitting 11 strains by *G. morsitans* and 8 strains by *G. palpalis*, the former strains including all the latter. The length of life of the monkeys is not given, but it may be supposed that enough monkeys died with striking rapidity to suggest that the cause was an increase in virulence of the strains of *T. gambiense* as a result of a single passage through *G. morsitans* in each case. Lester (1935) transmitted a strain of human trypanosomes through a series of 25 *G. tachinoides*, and the same strain, in a parallel experiment, through a series of 7 *G. submorsitans*, and found no significant difference between the trypanosomes of the two series of transmissions. He concluded that we have no proof that the species of fly plays any part in determining the characteristics of a trypanosome. Lester (1933) also examined 15 strains of human trypanosomes, 13 of which were Nigerian strains isolated from man a year or two previously. He concluded that ' the various characteristics which have been used to differentiate *T. rhodesiense* from *T. gambiense* appear altogether unsatisfactory,' and expressed his belief that the two trypanosomes are identical. At least ten years ago it was recommended that *T. gambiense* should be transmitted repeatedly by *G. morsitans* and *T. rhodesiense* by *G. palpalis* ; and it would seem to simplify the experiments if *T. gambiense* were obtained

in a country where it seems to be conveyed only by *G. palpalis*, and *T. rhodesiense* in a country where it seems to be conveyed only by tsetse-flies of the *G. morsitans* group. In countries where the areas of distribution of the two human trypanosomes and of their fly vectors show little, if any, overlapping, mixed infections of the human trypanosomes in flies and in man are likely to be few. All strains of human trypanosomes in Tanganyika Territory that have been examined since 1922 have shown, I think, the characters of *T. rhodesiense* in their virulence and nearly always also in the appearance of numerous posterior-nuclear forms in inoculated animals. In Uganda, also, it seems that all strains of human trypanosomes, from areas where *G. palpalis* is the only tsetse-fly, have shown the characters of *T. gambiense*. In the experiment recorded here, a strain of *T. gambiense* from Uganda has been transmitted by laboratory-bred *G. morsitans* for 21 months through a succession of monkeys of the genus *Cercopithecus*, a series of 8 transmissions having been made. The monkeys are a common kind, with black face and brownish-grey fur.

EXPERIMENT

In July, 1934, Mr. McDonald, acting for Dr. Duke, at the Human Trypanosomiasis Institute, Entebbe, kindly sent me a strain of *T. gambiense* in a monkey and two guinea-pigs. The monkey, m. 1292, had been infected by *G. palpalis* in April, 1934, from another monkey, m. 1282, which had been inoculated from a case of sleeping sickness on March 15th, 1934. The strain of *T. gambiense* had been found in Entebbe to have low virulence and infectivity, and was not easily transmitted there, inoculation of monkeys sometimes failing to infect them. Transmission by *G. morsitans* was begun about the middle of September, 1934, by feeding flies on monkey 1292, and was continued from monkey to monkey by boxes of flies without any attempt to isolate the flies. As a rule, from two to four boxes of flies, containing altogether about from 60 to 100 flies, were used for each attempt, and it sometimes failed. More flies were needed than are usually required for the transmission of *T. rhodesiense* at Tinde. The series of transmissions by flies is shown in the following scheme, the dates being the days on which boxes containing flies, supposed to be infected, were first applied to the uninfected monkey.



The monkeys were slow to show infection, and no infected salivary glands were found in dissections of nine lots of flies, but the rate of infection of the salivary glands must have been low.

PATHOGENICITY OF THE STRAIN

From November, 1935, examinations of thick blood-films of the monkeys were made, and other laboratory animals were inoculated from the monkeys in order to get some idea of the characters of the strain. The inoculations are shown in Table I and were made, in several instances, when trypanosomes were relatively numerous in the blood of the monkeys. Usually five or six drops of blood from a monkey's ear, in about an equal quantity of 1 per cent. saline, were inoculated, but sometimes it was possible to draw blood from a vein. During my absence on leave, two rats were inoculated from monkey 20 on April 29th, 1935, and one became infected and died on October 9th, 1935, presumably of the infection. A rough idea of the mild type of infection, in inoculated animals and in the monkeys infected by flies, is given by Tables II and III, which show the numbers of trypanosomes seen per 100 microscopic fields of stained thick blood-films, using a 1/12 inch oil-immersion objective and a 7 × eyepiece, and 200 fields being examined when the numbers were very few. Four of the monkeys have died, viz., m. 3, m. 13, m. 1292 and m. 15. Monkey 3 was inoculated from m. 1292 on August 21st, 1934, and died on October 14th, 1935; monkey 13 had suffered from dysentery in 1934 and died in January, 1935. Both of these monkeys were infected, but in neither case can it be certain that the animal died of trypanosomiasis. Monkey 1292 had become much thinner and weaker by April, 1936, and was chloroformed on May 2nd, when apparently dying. The cerebrospinal fluid contained 80 trypanosomes and 230 leucocytes per c.mm. Microfilariae were also present. There was pericardial effusion, the fluid containing red blood corpuscles but showing no trypanosomes. A little cerebrospinal fluid, 0·3 c.cm., was injected into rat 1869 and caused a chronic type of infection. Monkey 15 showed some loss of strength and activity for a few days before its death on June 27th, 1936. An attempt to obtain the cerebrospinal fluid, shortly after death, failed owing to puncture of a vein and contamination with blood. Pericardial effusion was present. The spleen weighed 21 gm., but the monkey had malaria. As these monkeys are hardy animals and death from other causes is very rare at Tinde, it is probable that monkey 15 died of trypanosomiasis. Posterior-nuclear forms were never seen in the blood of monkeys, but, as Table III shows, trypanosomes were never so numerous as to make the search for posterior-nuclear forms in thin blood-films easy. The differences between individual monkeys are also shown in Table III: monkeys 15 and 17 never showed many trypanosomes, while in monkeys 30 and 32 they were much more numerous. The blood of guinea-pig 353 showed so many trypanosomes, for about a week, as to resemble the appearance of an infection with *T. rhodesiense*. It was the only animal in which posterior-nuclear

TABLE I
List of inoculated animals

Infected animal	Inoculated animal	Date of inoculation	Result	Remarks
m. 1292	r. 1784	24.12.35	—	ear blood
,,	d. 3	27. 4.36	—	1 c.cm. vein
,,	r. 1869	2. 5.36	+	C.S.F.
m. 14	10 mice	23.11.35	—	1·5 c.cm. vein
,,	d. 5	28. 4.36	+	0·5 c.cm. vein
m. 17	10 mice	27.11.35	—	1·5 c.cm. vein
m. 18	d. 1	25. 4.36	—	2 c.cm. vein
m. 20	10 mice	23.11.35	—	1 c.cm. vein
m. 30	10 mice	23.11.35	—	1 c.cm. vein
,,	r. 1781	18.12.35	—	ear blood
m. 32	r. 1780	18.12.35	—	,,
,,	r. 1797	17. 1.36	—	,,
,,	gp. 340	17. 1.36	—	,,
,,	m. 41	21. 1.36	—	,,
,,	m. 39	7. 2.36	+	,,
m. 33	r. 1853	24. 3.36	—	,,
,,	gp. 352	15. 4.36	—	,,
,,	d. 4	28. 4.36	—	,,
m. 35	r. 1852	24. 3.36	—	,,
,,	gp. 350	15. 4.36	+	,,
,,	rbt. 14	25. 4.36	—	,,
m. 36	r. 1854	24. 3.36	—	,,
,,	d. 2	26. 4.36	—	,,
m. 40	gp. 351	15. 4.36	—	,,
,,	gp. 353	23. 4.36	+	2 c.cm. vein
,,	rbt. 15	29. 4.36	—	ear blood
,,	gp. 354	3. 5.36	—	½ c.cm. vein and ear
m. 43	gp. 349	15. 4.36	—	ear blood
gp. 353	gp. 355	3. 5.36	—	ear vein
,,	gp. 356	12. 5.36	—	,,
,,	gp. 357	14. 5.36	—	,,
,,	r. 1870	19. 5.36	+	,,
,,	m. 50	22. 5.36	+	,,
gp. 350	gp. 359	3. 6.36	—	,,
r. 1869	r. 1871(2)	23. 5.36	+	tail blood
,,	r. 1873	3. 6.36	+	,,

m. = monkey ; gp. = guinea-pig ; r. = white rat ; rbt. = rabbit ; d. = dog.

TABLE II

Course of infection in inoculated animals. Numbers of trypanosomes per 100 microscopic fields of stained thick films, with 1/12 inch objective and 7× eyepiece

Animal	m. 39	m. 50	gp. 350	gp. 353	dog 5	rat 1869	rat 1870	rat 1871	rat 1873
Date of inoculation	7.2.36	22.5.36	15.4.36	23.4.36	28.4.36	2.5.36	19.5.36	23.5.36	3.6.36
Inoculated from	m. 32	gp. 353	m. 35	m. 40	m. 14	m. 1292	gp. 353	rat 1869	rat 1869
Trypanosomes first seen on	18.3.36	21.6.36	23.5.36	1.5.36	17.6.36	22.5.36	16.6.36	14.6.36	21.6.36
18.3.36	1								
22.4.36	26								
1.5.36				15					
3.5.36				150					
5.5.36				35					
8.5.36				15					
9.5.36				11					
10.5.36				25					
11.5.36				27					
12.5.36				36					
13.5.36				100					
14.5.36				200					
15.5.36				500					
16.5.36				500					
17.5.36				200					
18.5.36				700					
19.5.36				1,500					
20.5.36				2,000					
21.5.36				1,500					

m. = monkey ; gp. = guinea-pig.

TABLE II—(*Continued*)

Animal	m. 39	m. 50	gp. 350	gp. 353	dog 5	rat 1869	rat 1870	rat 1871	rat 1873
Date of inoculation	7.2.36	22.5.36	15.4.36	23.4.36	28.4.36	2.5.36	19.5.36	23.5.36	3.6.36
Inoculated from	m. 32	gp. 353	m. 35	m. 40	m. 14	m. 1292	gp. 353	rat 1869	rat 1869
Trypanosomes first seen on	18.3.36	21.6.36	23.5.36	1.5.36	17.6.36	22.5.36	16.6.36	14.6.36	21.6.36
22.5.36				1,500		+			
23.5.36			+	2,000		+			
24.5.36			150			½			
25.5.36			10	1,000		0			
26.5.36				400		+			
28.5.36				0		+			
29.5.36			14	3					
30.5.36				16		10			
1.6.36				50		8			
2.6.36			25	66					
3.6.36			20	72		3			
4.6.36			13	60		5			
6.6.36				20		0			
8.6.36			10	18		0			
9.6.36						0			
10.6.36			14	20					
11.6.36						0			
12.6.36			2	14		4			
14.6.36						2		10	

m. = monkey ; gp. = guinea-pig.

TABLE II—(*Continued*)

Animal	m. 39	m. 50	gp. 350	gp. 353	dog 5	rat 1869	rat 1870	rat 1871	rat 1873
Date of inoculation	7.2.36	22.5.36	15.4.36	23.4.36	28.4.36	2.5.36	19.5.36	23.5.36	3.6.36
Inoculated from	m. 32	gp. 353	m. 35	m. 40	m. 14	m. 1292	gp. 353	rat 1869	rat 1869
Trypanosomes first seen on	18.3.36	21.6.36	23.5.36	1.5.36	17.6.36	22.5.36	16.6.36	14.6.36	21.6.36
16.6.36			38	28		0	4	14	
17.6.36					+				
18.6.36			6	7				0	
21.6.36		2		200	0	0	0	2	2
22.6.36		0				1	0		0
23.6.36			9	100				½	
24.6.36		1		200	1	1			½
25.6.36	3								½
26.6.36		2	60	20		3	0	1	0
27.6.36					0	2	0		½
28.6.36	2	4					0		
29.6.36			16	200			½	½	
30.6.36		26							
1.7.36					1				
2.7.36		11	36	200			8		
4.7.36		0	100	30	1	0	7	5	32
5.7.36			100				½		24
6.7.36		1½		62		0		0	1½

m. = monkey ; gp. = guinea-pig.

TABLE III

Course of infection in monkeys. Numbers of trypanosomes per 100 microscopic fields of stained thick films, with 1/12 inch objective and 7× eyepiece

Passage (*G.morsitans*)	—	1	1	2	3	4	5	6	6	7	7	7	8	8	8
Monkey	1292	15	18	14	17	20	30	32	36	35	33	40	43	45	48
Trypanosomes first seen on	3.5.34	26.10.34	27.10.34	11.1.35	26.1.35	18.3.35	3.10.35	20.11.35	19.1.36	7.2.36	25.2.36	12.4.36	14.4.36	28.4.36	4.6.36
21.11.35	0	0	+	+	0	+	+	+							
23.11.35	0	½			4		3	+							
25.11.35		0	12	4	0			0							
26.11.35	0					7	200	½							
30.11.35	0	1	100	½	0	3	50	200							
5.12.35	1	1	4	½	0	½	9	50							
7.12.35	12		4		½	1	50	100							
9.12.35	2		2	4	0	1	100	100							
13.12.35	5	1	4	4	½	20	20	20							
17.12.35	4	½	3	14	2	0	14	500							
21.12.35	0	½	26	16	1	0	8	14							
15. 1.36	200	2½	37	12	½	3	22	100							
18. 1.36	3	0	50	3	2½	25	9								

22. 1.36		2	50	9	2	6	2½	12	100						
2. 2.36		0	100	8	0	2	16	60	1						
7. 2.36	12	3	50	5	2	0	1½	50	2½	6					
13. 2.36	7	½	10	3	0	5	2	½	25	0					
25. 2.36	0	0	½	25	0	6	2	24	150		1				
18. 3.36	100	½	22	6	0	5	0	1	28	25	½				
23. 3.36										30	11				
12. 4.36												20			
14. 4.36													½		
15. 4.36										1	500	20	17		
22. 4.36	6	½	50	4	0	8	2	0	20	100	12	400	0		
25. 4.36			50							150		200			
26. 4.36									18						
27. 4.36	20														
28. 4.36				9		3	0	3	8	2	50	20	0	½	
29. 4.36												8		1	
3. 5.36												8			
8. 5.36									3	10	36	14	20	2	
11. 5.36					½					2	4	2	10	½	
14. 5.36					1½		1½	1	18	0	3	1		1	

TABLE III—(*Continued*)

Passage (*G.morsitans*)	—	1	1	2	3	4	5	6	6	7	7	7	8	8	8
Monkey	1292	15	18	14	17	20	30	32	36	35	33	40	43	45	48
Trypanosomes first seen on	3.5.34	26.10.34	27.10.34	11.1.35	26.1.35	18.3.35	3.10.35	20.11.35	19.1.36	7.2.36	25.2.36	12.4.36	14.4.36	28.4.36	4.6.36
21. 5.36										0	13		17	28	
4. 6.36															1
8. 6.36															6
12. 6.36															54
16. 6.36															4
24. 6.36			9	10	0			50							
25. 6.36		½				3									
26. 6.36							3		½	0	9	23	0	2	
28. 6.36			6	0	0			14		½			0	16	64
1. 7.36															29
2. 7.36															8

forms were seen; they were observed on two days, May 21st and 25th, one only being seen on the former date, but on May 25th they amounted to 5 per cent. of the total number of trypanosomes counted in a thin blood-film. Subsequent examinations of the blood of guinea-pig 353 and the infections in monkey 50 and rat 70 have not indicated that *T. gambiense* has become changed into *T. rhodesiense* in the guinea-pig. The results of inoculations of rats and dogs were uncertain, and when infection appeared it had a mild course. Although it would be interesting to know whether the strain is still infective to man, it has not so far been thought justifiable to ask for a volunteer, owing to the low virulence of the strain which might make the diagnosis of infection difficult. If, however, 'Bayer 205' were given after about a fortnight, if no trypanosomes were found in the volunteer's blood, the risk would probably be removed. Except in guinea-pig 353 the incubation periods have been long.

SUMMARY

A strain of *T. gambiense* was transmitted by *G. morsitans* through monkeys. So far as could be judged by the course of the infection in the monkeys, and by the results of inoculations of laboratory animals, no increase in virulence took place during eight passages.

COMMENTS

As it has been shown experimentally that each of the polymorphic trypanosomes can be conveyed by more than one species of tsetse-fly, and as African natives travel so much within their own country, it does not seem likely that the peculiar geographical distribution of the trypanosomes and their association with certain tsetse-flies will be explained until at least one of the species of trypanosome has been changed experimentally into one of the other two, if that is possible. If this could be brought about in a laboratory, no matter under what experimental conditions, it would suggest that the change might occur in nature. Observations of this strain of *T. gambiense* in various animals will therefore be continued, but a more virulent strain of *T. gambiense* from an unimpeachable source, if that is possible, might offer a greater chance of success and be less difficult to work with.

In a country where sleeping sickness exists, we cannot now be sure that any strain of polymorphic trypanosomes, obtained from an animal or from wild tsetse-flies, has not infected man within a few years before. Strains of *T. brucei*, therefore, should be obtained in a country where sleeping sickness is not known to occur. In such experiments the acquisition of characters would, of course, be more convincing than their apparent loss.

In a country where *T. gambiense* and *T. rhodesiense* occur together, it may be

necessary to consider the possibility of the occurrence of mixed infections when examining the characters of any strain of human trypanosomes.

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RABIES IN THE VAMPIRE BAT OF TRINIDAD, WITH SPECIAL REFERENCE TO THE CLINICAL COURSE AND THE LATENCY OF INFECTION

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Hurst and Pawan (1931, 1932) and Pawan (1936) have shown that in Trinidad blood-lapping vampire bats (*Desmodus rotundus murinus* Wagner, 1840) are the transmitting agent in the spread of paralytic rabies among animals and human beings. The presence of Negri bodies in certain bats, the experimental production of the disease in animals by the use of infected material from bats, and the clinical histories of persons who developed the disease after being bitten, justify the conclusion that vampires infected with rabies are the vector of infection to man and animal. The disease occurs in man, in the herbivorous mammals, such as cattle, horses, sheep, goats, donkeys, etc., and in blood-lapping and non-lapping bats; the blood-lapping vampire, by reason of its special peculiarity of feeding on blood and the consequent necessity of inflicting a wound to obtain its meal, readily spreads infection. The clinical manifestations of the disease, as it affects these animals and man in Trinidad, have been carefully studied, but the wild, nocturnal habits of the vampire rendered observation in captivity difficult. The discovery, however, by Clark and Dunn (1932), as reported by Ditmars and Greenhall (1935), that blood-lapping *Desmodus* bats can survive for prolonged periods on defibrinated blood, made it possible to examine under laboratory conditions the clinical course of the disease, and to determine the effects of infection upon the natural habits of these strange mammals.

At the beginning of the outbreak of paralytic rabies in Brazil in 1908, the inhabitants of Santa Catharina and St. Pedro d'Alcantara remarked that, though bats are normally nocturnal animals, many were seen flying about during the day and biting animals in bright sunshine. Carini (1911), who had proved the infection to be due to rabies, emphasized the fact that bovines bitten by such bats invariably developed the disease, but he was unfortunately unable to obtain specimens for examination. Haupt and Rehaag (1914-21) in Blumenau, South Brazil, were also impressed by the unusual spectacle of bats flying during the day and shrieking and fighting with one another, and concluded that ' such abnormal aggressiveness and biting fits must surely be regarded as signs of disease.' Examination of such bats proved them to be infected with rabies. On the other hand, Lima (1934) demonstrated in Matto Grosso, Brazil, that *Desmodus* bats, after artificial inoculation with the virus of rabies, may remain healthy, without any evidence of disease, and yet be able to transmit infection to bovines by their

bites for as long as 4½ months after infection ; further, even in areas where the disease is not occurring, *Desmodus*, caught under natural conditions and apparently healthy, may harbour the virus in their brains and salivary glands. Torres and Lima (1934), working in Santa Catharina and Blumenau, Brazil, also found bats infected in nature, both in areas where the disease was prevalent and in supposedly non-infected areas. Such infected bats, though evincing no abnormal symptoms, were shown capable of producing rabies by biting bovines. They studied the clinical manifestations and duration of infectivity of the disease in bats, and the incubation-period in animals bitten under laboratory conditions. They were also able to produce the disease by the artificial inoculation of bats, and animals bitten by such bats developed rabies subsequently. Bats, they concluded, may become carriers of rabies under both natural and artificial conditions, and may remain so for over three months without manifesting any abnormality.

In Trinidad, during the early period of the outbreak of paralytic rabies, authentic accounts were received of bats flying and biting animals during the day, and actually fighting with one another and with cats, goats and dogs in their attempts to bite ; these sights were unusual within the memory of the present generation of inhabitants. Examination of such bats proved them to be infected. The conclusion was therefore drawn that bats infected with rabies exhibit these abnormal manifestations. Soon, however, in the course of the routine examination of bats captured under natural conditions, it was discovered, both by histological study and by animal inoculation, that bats may harbour infection and yet show no signs of disease nor any abnormality in habit. In one instance, a bat caught at Guanapo village and kept in captivity for 5½ months was found to be harbouring the virus in its brain. Meanwhile, reports as to the unusual phenomenon of bats fighting and biting during the day ceased. It therefore became necessary to verify the thesis that rabid bats exhibit abnormal habits, and at the same time to study, even though under artificial conditions, the clinical manifestations of the disease in infected ones, especially as the confirmation of the belief that only bats showing some abnormal habit are infectious and should be destroyed would facilitate the control and prevention of the disease.

MATERIALS AND METHODS

Cases of paralytic rabies have occurred among animals in almost every part of the colony, and, with the knowledge that bats are the vector of the disease, the difficulty arose of providing bats, especially *Desmodus*, free from infection for study. To secure this end, they were captured from supposedly non-infected areas and allowed, whenever possible, to feed in a bat-proof stall on a calf, also from a non-infected area, or on a rabbit. The calf or rabbit after being bitten (usually on more than one occasion) was then removed and kept in confinement under ' bat-proof ' conditions. If it developed rabies, it was assumed that one or more of the bats had been infected. Rabbits proved unsuitable,

as a vampire would usually bleed a rabbit to death during a night. On the other hand, through financial reasons it was not possible to allow each and every bat to feed upon a calf. The bats were kept in bat-proof cages about 10 × 10 × 8 inches in size, the vampires being fed nightly with defibrinated ox blood in Petri dishes, and the *Artibeus* with fruits. For artificial infection, they were inoculated by the subcutaneous route, from 0·1 to 0·5 c.cm. of a 10 per cent. brain emulsion in normal saline being given in the tissues of the dorsal region with a hypodermic needle and syringe. In the case of the rabbit, unless otherwise stated, the injections were given intracerebrally with a dose of 0·2 c.cm. of a similar emulsion. For the detection of infection in the saliva of the dead bat, a sterile swab was rubbed over the tongue and mucous membrane of the mouth and then immediately applied over the scarified abdominal wall of the rabbit, while the live bat was allowed to feed on the animal to be infected. The human strain of virus used in this investigation was isolated from a human case (V.J.), in March, 1934, and having been passaged repeatedly in rabbits had lost its negrigenic power. The bat strains were obtained from the bats indicated. The virus was preserved for varying periods in equal parts of glycerine and saline at a temperature of 0–5° C. For the detection of Negri bodies smears and sections were made from the hippocampus. The smears were stained by Van Gieson, Gerlach and Muromzew methylene blue-tannin methods. The sections were fixed in Zenker, dehydrated with dioxane, and stained by Mann's method. If Negri bodies were not seen in the hippocampus, in many instances 0·2 c.cm. of a 10 per cent. emulsion of the brain or left sciatic nerve was injected into the right sciatic nerve, and this was again repeated, if necessary, to stimulate the formation of Negri bodies as devised by Nicolau and Kopciowska (1935). The following are the details of some of the experiments undertaken.

ARTIFICIAL INOCULATION

Experiment No. 1

On October 17th, 1935, six *Desmodus* bats (nos. 1, 2, 5, 9, 11, 12) were caught at Gasparillo Cave, Santa Cruz, and allowed to feed from the night of October 21st–25th on a calf 3 weeks old. The calf (no. 1) was bitten on the night of the 21st–22nd on both front legs and on the nose; on the 22nd–23rd on the nose, left shoulder, left front and hind legs, and right flank; on the 23rd–24th on the right shoulder and left hind leg; on the 24th–25th on the right shoulder and the left hind leg. On the morning of the 25th it was removed to a 'bat-proof' stable for observation.

On the 25th, each bat was injected with 0·1 c.cm. of an emulsion of the brain of a rabbit which had died on that same day after infection with the human virus.

The following day bat 1 died. Histological examination showed no Negri bodies. On November 5th, i.e., 11 days after infection, another bat, no. 2, died without any evidence of disease before death. The hippocampus showed

doubtful Negri bodies. A portion of the brain was injected into a rabbit on November 6th. Paralysis developed on the 23rd, and death took place on the 24th, i.e., in 18 days. Negri bodies were present in the hippocampus. From this latter rabbit another was inoculated intra-ocularly on November 26th. Death with paralysis took place on December 8th, i.e., in 12 days, and the hippocampus showed Negri bodies.

On November 25th–26th, bat 5, which so far had shown no sign of disease, was allowed to feed on a rabbit (r. 1), the hair over the back having been clipped. The bat bit the rabbit on the hind leg, inflicting on oval superficial wound about 2 mm. long. On January 6th, i.e., 41 days later, the rabbit was paralysed, and death took place on the following day. Doubtful Negri bodies were seen. On the 6th the sciatic nerve of rabbit 1 was injected into the sciatic nerve of rabbit 38, and into the brain of another, no. 40. The former died in 18 days, and the hippocampus showed Negri bodies ; the latter died in 35 days, but no Negri bodies could be seen.

On November 29th, bat 5 was again placed in a cage with a rabbit at 9.45 a.m. It had fed the previous night on defibrinated blood. At about 11.30 a.m. it bit the rabbit on the right hind leg, and on the same spot and on the nose during the night of November 30th–December 1st. On the morning of December 1st, the rabbit was found dead.

Up to January 6th, bat 5 was well. On the 7th, it was seen to be weak and unable to fly, and rested on all four limbs on the floor of the cage, the thumbs being lacerated, probably from earlier attempts to cling to the wire of the cage. When a stick was brought against its body, it was unable to move or crawl, though at times it would bite ' viciously ' at the stick. There was no salivation and no incontinence of urine. On the morning of the 8th, it was found dead, i.e., 2 months and 13 days after infection. The hippocampus showed Negri bodies. On the day of death the saliva was rubbed into the scarified abdominal wall of a rabbit, which, however, died the following day.

On January 6th, 1936, i.e., 2 months and 12 days after infection, bat 9 was found dead, without having shown any previous evidence of illness. The hippocampus showed numerous Negri bodies. An emulsion of the brain was injected into rabbit 39 on January 7th. Death took place on the 21st, i.e., in 14 days, and the hippocampus also showed numerous Negri bodies. Three hours after death on January 6th, the saliva of bat 9 was rubbed into rabbit 37. On January 25th, i.e., 19 days later, the rabbit was paralysed, and death took place on the 27th, i.e., in 21 days. The hippocampus showed Negri bodies. The salivary glands were also removed from bat 9 on January 6th, and on the following day an emulsion was injected into rabbit 40. On the 25th, paralysis appeared, and death took place on the 29th, i.e., in 22 days. The hippocampus was unfortunately not examined.

During the night of January 14th–15th, 1936, bat 11 was caged with rabbit 49. The rabbit was bitten on the ear and was removed on the morning of the

15th. On the 28th, there was paralysis of the hind quarters, and death took place the following day, i.e., in 13 days. The hippocampus showed Negri bodies.

During the night of February 17th–18th, 1936, another rabbit, no. 69, was caged with the same bat 11. It was bitten on the right ear and was removed to the laboratory on the morning of the 18th. On the 27th, i.e., in 9 days, it was found dead, but no Negri bodies could be seen.

On April 8th, 1936, i.e., 5 months and 14 days after infection, bat 11, without having shown any evidence of disease, was found dead. Smear preparations of the hippocampus showed Negri bodies. On the following day the saliva was applied to the scarified abdominal wall of rabbit 128. Death with emaciation took place on the 27th, i.e., in 18 days, and Negri bodies were present in the hippocampus.

On December 7th, 1935, six fruit-eating bats (*Artibeus planirostris trinitatis*), caught in the city of Port-of-Spain, were caged with *Desmodus* bat no. 12. Five died in the following three days, one with recent bites on the left forearm and chest. The sixth remained with the vampire for 7 days, after which it was removed to a separate cage. Up to February 7th, i.e., 1 month and 24 days after separation from the vampire, it showed no evidence of disease. It was killed on that day, and the saliva was smeared into rabbit 58. Negri bodies were not seen in its hippocampus. On February 21st, 14 days later, rabbit 58 died with paralysis, but Negri bodies were still not seen in sections of the hippocampus.

On January 14th, 1936, five fruit-eating *Artibeus* bats were again caged with *Desmodus* bat 12. On February 10th, i.e., after 27 days, three of them were removed and killed, and their saliva was rubbed into one rabbit, no. 64. Neither the *Desmodus* nor the *Artibeus* had shown any sign of disease. On February 21st, i.e., 11 days later, rabbit 64 died. It was emaciated, but Negri bodies were not seen in sections. On the same day, an emulsion of the brain was injected into the right sciatic nerve of guinea-pig 74. Death took place in 14 days, but Negri bodies could not be found. A portion of the left sciatic nerve of guinea-pig 74 was injected on March 7th into the right sciatic nerve of another guinea-pig, no. 84. Death took place in 23 days, but still no Negri bodies were seen in the hippocampus. On March 31st an emulsion of the left sciatic nerve of guinea-pig 84 was injected into the right sciatic nerve of guinea-pig 108. Death followed in 4 weeks' time, but still without Negri bodies.

From November 30th–December 3rd, 1935, bat 12 was caged with a two-weeks-old calf. The calf was not bitten on November 30th–December 1st; on the 1st–2nd it was bitten on the neck; on the 2nd–3rd on the left leg; and it was removed on the morning of the 3rd. It remained well until May 8th, 1936, when it was found paralysed in both hind quarters, and death occurred the following day, i.e., 5 months and 9 days after being bitten. Sections of the hippocampus showed Negri bodies.

On December 24th, another two-weeks-old bull calf, no. 4, was caged with bat 12. He was bitten during the night of the 25th–26th and of the 27th on the nose, and was removed on the morning of the 28th. On April 27th, 1936, i.e., 4 months later, he was found lying paralysed on the left side of the body. On the 28th he was able to rise, but would sway from side to side in his attempt to walk, and there was profuse salivation. On the 29th the calf was again prostrated and completely paralysed. He was slaughtered in the afternoon, but no Negri bodies were seen. On May 1st an emulsion of the brain was injected into the sciatic nerve of rabbit 132. Paralysis developed on the 12th, and death took place on the 13th, i.e., in 12 days. The hippocampus showed Negri bodies.

On February 14th, bat 12 died, i.e., 3 months and 21 days after inoculation. During that period it never at any time showed any signs of disease. The carcass was kept at a temperature of 5° C. until the 17th, when the saliva was smeared over the abdomen of rabbit 68. Death took place on the 28th; the rabbit was emaciated, but no Negri bodies could be seen. The hippocampus of bat 12 also showed no Negri bodies.

On March 20th, 1936, i.e., about 5 months after being bitten by these bats, calf no. 1, which was referred to above and which was employed to detect possible infection in the vampires, was seen to be paralysed in the hind quarters and unable to rise. Death took place the following day, and the hippocampus showed Negri bodies. It was not possible to determine which bats were infected before inoculation.

Experiment No. 2

Six *Desmodus* bats (nos. 3, 4, 6, 7, 8, 10), captured at Nelson Bamboo, St. Joseph, on October 2nd, 1935, were allowed to feed on a young calf (no. 2) from the night of October 29th to November 1st. The calf was bitten on the 29th–30th on both hind legs and on the right flank; on the 30th–31st on the left shoulder, front leg, neck, flank and hind leg; on October 31st–November 1st over the body. On the 1st the calf was removed to a bat-proof stall for observation.

On November 8th, each bat was given subcutaneously 0·2 c.cm. of a brain emulsion of the human virus.

On November 17th, i.e., 9 days later, bat 3 died. It had been recently bitten by the other bats on the head and left shoulder. Sections of the hippocampus showed Negri bodies. On the 18th an emulsion of the brain was injected into a rabbit. Ten days later paralysis appeared in the left hind legs, and death took place on November 29th. Sections of the hippocampus also showed Negri bodies.

On November 25th, i.e., 17 days after inoculation, bat 4 was found lying on the floor of the cage unable to fly. When touched with a stick she would fall forward on to the chest, but would make no attempt to bite or show any sign of violence. Closer examination revealed a purulent conjunctivitis of

both eyes, and a definite paralysis of the left hind limb and both fore limbs, and also of the lower jaw and neck muscles. She was drowsy and apathetic. Later in that day there was incontinence of urine. The following morning the paralysis of the hind limb was more marked, and there was much incontinence of urine ; she was also distinctly ' vicious.' If a stick was placed near to her she would snap fiercely at it. On the 27th, 28th and 29th she became weaker and was quite helpless in the day, though still snapping and attempting to bite. Being obviously moribund she was killed on November 29th. Sections of the hippocampus showed Negri bodies. An emulsion of the brain was injected on December 4th into a rabbit (r. 1). Paralysis of the right side of the body appeared on the 13th, and death took place on the 15th, i.e., 11 days later, but no Negri bodies could be seen. A brain emulsion of rabbit 1 was injected into the right sciatic nerve of another rabbit, r. 5, on the 16th. Death took place on December 27th, but no Negri bodies were seen. The left sciatic nerve of rabbit 5 was injected intracerebrally on the 27th into rabbit 19, and into the right sciatic nerve of rabbit 20. The former died on January 5th, i.e., in 9 days, and Negri bodies were seen, while the latter died on February 11th, and no Negri bodies were seen. On January 6th the left sciatic nerve of rabbit 19 was injected into the right sciatic nerve of rabbit 35. Death took place in 8 days, but still without Negri bodies.

On November 25th, 1935, when bat 4 was first seen to be paralysed, a dry stick was inserted through the wire mesh of the cage and into her mouth. Careful examination showed the stick to be *just moist*. It was then immediately rubbed into the scarified abdominal area of rabbit 7. Paralysis appeared in both hind legs on December 2nd, i.e., 7 days later, and death took place on the 3rd. Negri bodies were present in the hippocampus.

On December 3rd, 1935, i.e., 25 days after injection, bat 6 was found dead. It had shown no sign of disease and no abnormality. Sections of the hippocampus revealed Negri bodies. The salivary glands were dissected out on the day of death, and an emulsion was injected into rabbit 18 on the 4th. An emulsion of the brain was also injected on the same day into another rabbit. Rabbit 18 developed paralysis and died on January 10th, i.e., 1 month and 6 days later ; and the second rabbit died on December 20th, i.e., in 16 days. Negri bodies were not seen in either rabbit.

On November 30th, 1935, bat 7, which had fed the previous night, was caged with a calf. Biting did not take place until during the *day* of December 2nd, between 2 p.m. and 4 p.m., when the calf was bitten on the neck. On the morning of the 3rd it was removed. The calf was therefore bitten once only and during the day, 24 days after the *Desmodus* was injected. On March 9th, i.e., 3 months and 7 days after being bitten, the calf, by then grown thin, showed definite paresis of the hind quarters. It would sway from side to side when made to walk, and there was a definite keratitis. Death took place the following day. The hippocampus showed no Negri bodies.

On December 7th, 1935, five fruit-eating (*Artibeus*) bats, caught on the 5th of the same month in the city of Port-of-Spain, were caged with bat 7. One died on December 8th, another on the 14th, and two on the 16th. None of them showed Negri bodies, but there were bites over their bodies. The fifth and last died on December 16th. Previous to death it had shown no abnormality, but sections of the hippocampus showed numerous beautifully stained Negri bodies. On December 20th the brain was injected into rabbit 11. Paralysis appeared in 11 days, and death took place the following day, but no Negri bodies were seen. From this, further inoculations were made on January 3rd intracerebrally into rabbit 28, and intrasciatically into rabbit 29. Death took place in 12 and 8 days respectively. Negri bodies were present in rabbit 29, but were absent in no. 28.

On December 16th, bat 7, which had previously been well, was found helpless on the floor of the cage, paralysed in all four limbs. It could not fly or move, and it died that same day, i.e., 1 month and 8 days after inoculation. The hippocampus showed numerous beautifully stained Negri bodies. At 4 p.m. that day the saliva was smeared into rabbit 9; death with paralysis took place in two days' time; the brain was not examined. An emulsion of the brain of bat 7 was, however, injected into another rabbit on December 18th. Death with paralysis took place on January 3rd, 1936, i.e., in 16 days, and doubtful Negri bodies were seen in the hippocampus. Further inoculation was made on the same day into guinea-pig 34. Death took place on February 24th, and no Negri bodies could be seen. The left sciatic nerve of guinea-pig 34 was then injected intrasciatically into another guinea-pig, no. 76, on February 27th. Death took place on March 6th, i.e., in 7 days, and the hippocampus showed Negri bodies.

On December 16th, 1935, bat 8, which had so far shown no abnormality, developed a definite paresis of the wing muscles and of all four limbs. It was unable to fly or walk, and there was incontinence of urine. There was no unusual desire to bite, no aggressiveness and no salivation, but there was a keratitis, with a purulent conjunctivitis. Death took place in the night of December 16th–17th, i.e., 5 weeks after infection. The hippocampus showed Negri bodies, but not many. The brain was injected into rabbit 8, on the 18th. Death with paralysis took place on the 31st, i.e., in 13 days, and doubtful Negri bodies were present. The left sciatic nerve of rabbit 8 was, on the same day, injected intracerebrally into rabbit 27 and intrasciatically into rabbit 26. Rabbit 27 died on January 19th, i.e., in 19 days, and showed small-size Negri bodies; and rabbit 26 died on January 21st, i.e., 3 weeks later, and showed Negri bodies. The sciatic nerve of rabbit 26 was further injected into the right sciatic nerve of rabbit 56 on January 21st. Death took place on February 13th, i.e., in 23 days, and numerous Negri bodies were present in the hippocampus.

During the night of January 13th–14th, 1936, bat 10 did not feed well.

On the morning of the 14th it was seen to be paralysed in the right fore limb and was unable to fly. There was incontinence of urine, and it was ' vicious ' and would bite fiercely at any object brought within its reach. It was then placed in a cage with two other vampires captured from a supposedly non-infected area. The following day the paralysis had not advanced, but the bat was still very fierce and would bite furiously at a stick placed near its mouth. There were marks of bites inflicted by other bats. It died on January 18th, i.e., 2 months and 10 days after infection. The hippocampus showed numerous Negri bodies of all sizes, large, medium and small ; they were more numerous than in any other bat. Two hours after death the saliva was rubbed over the scarified abdomen of rabbit 52 and the scarified back of rabbit 57. They died on February 18th, i.e., 4 weeks later, and Negri bodies were present in the hippocampus in both instances.

The calf, no. 2, on which these 6 bats had fed from October 29th to November 1st, 1935, before they were inoculated, remained alive and well up to the date of writing, July 31st, 1936.

Experiment No. 3

On February 7th, 1936, four *Desmodus* bats, captured at Point Gourde on December 21st, and one rabbit were each injected with 0·2 c.cm. of a 10 per cent. emulsion of the brain of a rabbit infected with the human virus. The brain had been in glycerine saline at 0–5° C. for 17 days. On February 20th, i.e., 13 days later, the rabbit died with paralysis. The vampires were kept in separate cages and remained quite well, without any evidence of disease. On April 4th, 1936, i.e., about 2 months after the first inoculation, each of these four bats was again injected with 0·3 c.cm. of an emulsion of the same virus from another freshly infected rabbit brain, which had been in glycerine saline for 2 days at a similar temperature. Again they remained perfectly well, without any evidence of disease. On April 17th, they were given 0·6 c.cm. of a similar emulsion of the same human virus from rabbit 115, which had been injected *subcutaneously* on April 9th, 1936, and had died with paralysis on April 16th, showing Negri bodies in the hippocampus. All these four bats remained well, without any abnormality, after three different injections. On May 11th they were allowed to feed on a calf, three weeks old, obtained from the Farm, St. Joseph. On May 11th–12th, the calf was bitten on the back ; on the 12th–13th on the same spot on the back ; and on the 13th–14th again on the same spot. There was no biting on the 14th–15th. On the 15th–16th he was bitten in two places, again on the back, and he was removed on the morning of the 16th. There was gradual loss of flesh, with paresis of the hind quarters, on June 2nd, and death occurred the following day, i.e., 23 days after the first bite, but no Negri bodies were seen in the hippocampus. On June 8th a brain emulsion from this calf was injected intra-ocularly into rabbit 145. Death took place in 7 days, and the hippocampus showed Negri bodies. On the 3rd of the same month another three-

weeks-old calf, which had been kept in a bat-proof stall from birth, was placed with these four bats. Between the 3rd–4th the calf was bitten on the left hind leg and the tail ; on the 4th–5th on the nose, neck and shoulder ; on the 5th–6th on the left hind leg and tail ; on the 6th–7th on the right foreleg and back ; on the 7th–8th on the back and tail ; and on the 8th–9th on the left foreleg and nose. On the morning of the 9th he was removed. There was gradual loss of flesh, and on July 3rd, i.e., 28 days after being first bitten, paralysis appeared in the hind quarters, and death took place the following day ; but the hippocampus showed no Negri bodies. On May 20th two young goats were stabled with these four bats. One was bitten between the 20th–21st on the tail and on the right hind leg, and between the 21st–22nd on the right knee and on the right foreleg. The other was bitten only between the 20th–21st on the right hind leg. They were removed on the morning of the 21st, and have remained well up to the date of writing, July 31st, 1936.

Experiment No. 4

On February 28th, 1936, four *Desmodus* bats, also captured from Point Gourde on the 17th of the same month, were each injected with 0·3–0·4 c.cm. of a 10 per cent. emulsion of a bat virus known as 2799/2. This strain of virus was obtained from a bat caught in Sangre Grande on October 5th, 1935, and had been passaged nine times by intrasciatic inoculation and had recovered its negrigenic power.

On March 12th, i.e., 14 days after injection, bat 1 was seen to be fierce and ' vicious.' If anyone approached the cage—even without touching it—he would fly violently towards the wire netting and would lacerate his face and wings in attempts to get through the mesh ; he would snap and bite at the wire, or jump violently from one side of the cage to the other. At other times he would hang head downwards with lips retracted, snarling and staring fixedly at the observer. If a stick was brought through the wire mesh into the cage he would dart at it, and bite and gnaw with a hissing sound. The symptoms of fury shown by this bat were more marked than in any other, and can be truly described as the ' furious form of rabies.' There was no evidence of paresis or incoordination of movement. On the same day when these symptoms were noticed, i.e., March 12th, he was let loose in a cage with a young goat. He did not bite in the night of the 12th, but on the 13th–14th he bit on the left foreleg, on the 15th–16th on the right shoulder, and on the 16th–17th again on the same spot on the right shoulder. On the morning of the 17th the goat was removed. From the 15th the bat seemed less fierce and ' vicious,' and on the 17th was normal in manner and appearance. On April 8th, i.e., 25 days after the first bite, the goat died. It was very thin, and the hippocampus showed numerous Negri bodies. A portion of the brain was injected into rabbit 116 on April 12th. Death with paralysis took place in 8 days, and the hippocampus showed numerous Negri bodies. Bat 1 has remained alive and well, and is

normal in habits and appearance, without evidence of ' fury.' The other three bats are also alive and well.

Experiment No. 5

On March 6th, 1936, four *Desmodus* bats (A, B, C, D), captured on the 17th of the previous month, also from Point Gourde, were each injected with 0·4 c.cm. of a 10 per cent. emulsion of the brain of a guinea-pig which had died on March 4th, after intracerebral infection with the human virus.

On the 17th, i.e., 11 days after inoculation, bat A showed paresis of the hind quarters and right wing. On the following day it was found lying helpless on the floor of the cage, on the left side of the body, unable to move, with its eyes almost completely closed. It could not lift the left wing or the left leg, nor turn to one side. It could, however, raise the head slightly and could move the right wing and right leg. There was therefore complete paralysis of the left wing and leg, but only paresis of the right wing and right leg, though the right wing was first involved. On the morning of March 19th, i.e., 13 days after infection, it was found dead. Sections of the hippocampus showed Negri bodies.

On the morning of the 18th, i.e., 12 days after infection, bat C was also found helpless on the floor of the cage, with complete paralysis of the wings and right leg, and paresis of the left leg. It was lying flat on the abdomen, slightly on the left side and forward on the chest. There was also a keratitis. When thrown on to its back it would attempt fruitlessly to regain its former position. The following morning, i.e., 13 days after infection, it was found dead. The hippocampus showed Negri bodies.

In the afternoon of March 18th, i.e., 12 days after infection, *Desmodus* B was seen resting helpless on the floor of the cage, with complete paralysis of the wings and paresis of the legs. The following morning it was found dead. The hippocampus also showed Negri bodies. Bat D is alive and well.

Experiment No. 6

On April 4th, 1936, three vampires (A, B, C), captured at Scotland Bay two days previously, were each injected with 0·3 c.cm. of a brain emulsion of a bat strain 2799, which had been passaged 6 times intrasciatically through rabbits. Three days after inoculation bat A seemed easily excited, flying about the cage if anyone approached. Towards the afternoon, however, he was normal. On the 11th, i.e., 7 days after infection, he was more easily excited and was distinctly fierce. He would remain quiet in a corner of the cage, and then would suddenly fly violently against the wire netting, snapping and biting at it. On the 14th he was again quiet and normal, but in the afternoon at 4 p.m. he was found dead, i.e., 10 days after infection. There was never any evidence of paralysis. Sections of the hippocampus showed Negri bodies. The other two bats are alive and well.

Experiment No. 7

On April 15th, 1936, three *Desmodus* bats, captured at Barrackpore on March 11th, 1936, and kept in captivity on defibrinated blood for over 4 weeks, were each injected with 0·4 c.cm. of a 10 per cent. emulsion of the brain and cord of bat A of experiment 6. They have remained alive and well up to the date of writing, July 31st, 1936.

Experiment No. 8

On April 15th, 1936, three other *Desmodus* bats, caught at Couva on the previous day, were each injected with 0·4 c.cm. of the same emulsion of the same bat strain of virus. During the night of the 15th–16th one of them aborted, and died shortly after. The brain was unfortunately not examined. The other two have remained alive and well up to the date of writing, July 31st, 1936.

INFECTED BATS CAUGHT IN NATURAL CONDITIONS

1. On February 18th, 1936, a *Desmodus* bat, no. 6173, was captured hanging alone in an underground drain at St. James, a near suburb of the city of Port-of-Spain. It was left without food that day and during the night of the 18th–19th. It showed no abnormality, except that it was captured as a solitary inhabitant of the drain. In the course of the examination of bats captured in nature from various parts of the colony, it had been observed that the solitary bat frequently showed Negri bodies. It was therefore felt that this bat caught alone in this underground drain might be infected. On the morning of February 19th, at 10 a.m., it was caged with a two-weeks-old calf, which had been pastured during the day and stabled in a bat-proof stall at night. The bat was a female and was pregnant. It seemed as if there was some incontinence of urine, but otherwise she was apparently quite well and healthy. During the night of the 19th–20th the calf was bitten on the right foreleg ; on the 20th–21st on the left shoulder ; on the 21st–22nd on the nape of neck ; it was not bitten on the 22nd–23rd. On the 23rd–24th it was again bitten on the nape of the neck, and also on the 24th–25th ; on the 25th–26th it was bitten on the left shoulder, and on the 26th–27th again on the neck ; on the 27th–28th it was bitten on the old wound on the neck. On February 28th–March 1st, no record was kept. On March 1st–2nd the calf was not bitten, and he was removed on the morning of the 2nd to a bat-proof stall. He gradually became thin, and died on March 12th, 1936, i.e., about 20 days after being first bitten. Numerous Negri bodies were seen in both smears and sections of the hippocampus. A brain emulsion was injected into rabbit 89 on the 12th. Death took place 4 weeks later, and the hippocampus showed Negri bodies.

On March 12th, 1936, a young goat obtained from a non-infected area was caged with this same bat, no. 6173. The kid was bitten during the night of the 12th–13th on the left foreleg; on the 13th–14th on the same leg near the hoof; on the 15th–16th on the left hip; it was not bitten on the 16th–17th, and no record was made for the 14th–15th. On the morning of the 17th the goat was removed. It has remained well up to the date of writing, July 31st, 1936.

On the day (March 17th) when the goat was removed, another calf was placed with bat 6173. On the 17th–18th the calf was bitten on the neck, left foreleg and back; on the 18th–19th on the neck; on the 19th–20th on the left hip; on the 20th–21st he was not bitten; on the 21st–22nd he was bitten on the neck; and on the 22nd–23rd on the right hind leg. There was progressive loss of flesh, and on April 28th the calf was paralysed on the left side of the body. The following day the paralysis was still present, but he was able to eat. Towards the afternoon he was moribund and was therefore slaughtered the same day, i.e., 5 weeks after being first bitten. The hippocampus showed no Negri bodies. A portion of the brain was injected into the sciatic nerve of rabbit 133 on May 1st. Death took place in 5 days' time, and Negri bodies were present in the hippocampus.

During the night of April 2nd–3rd, a full-grown donkey was caged with bat 6173. It was bitten on the left shoulder and was removed for observation. It has remained well up to the date of writing, July 31st, 1936.

On the morning of April 15th, bat 6173, which had been healthy and well, without any evidence of illness, was found dead. As mentioned above, she was a pregnant female, and the head of the foetus was impacted in the vagina. The hippocampus showed numerous Negri bodies.

2. On March 19th, 1936, 90 bats were caught in a hollow silk-cotton tree at Sangre Grande. They were brought about 20 miles to the laboratory in three cages, 20 to 30 bats being in one cage. It was noticed that no. 9916 was unusually excited and restless. It was removed into a separate cage, together with two others, but the restlessness still continued. It would dart repeatedly from one side of the cage to the other; it would remain hanging head downwards, grinning and uttering hissing cries if anyone approached the cage; or it would fly violently against the wire bars of the cage towards the observer. It was removed into solitary confinement, but continued to dart about restlessly in its cage, splashing the defibrinated blood which had been placed in the Petri dish as its food. On the following day, the 20th, paresis of both wings and the left leg was noticed, the right leg being normal. With the right leg the bat was supporting itself from the roof of the cage, the head hanging downwards. Towards the afternoon of the 20th there was complete paralysis of the wings and legs, and it was unable to fly, walk or suspend itself; it was lying still on the floor of the cage, looking helplessly at the observer, with all its energy and violence gone. At about 4 p.m. it was clearly moribund, and in the morning of the 21st was found dead. Towards the afternoon of that day the saliva

was rubbed into the scarified abdominal and dorsal areas of a full-grown *Macacus rhesus* monkey, which has remained alive and well up to the date of writing, July 31st, 1936. The hippocampus of bat 9916 showed, however, numerous Negri bodies. Further inoculation was not undertaken.

3. On April 25th, 1936, in a batch of 75 *Desmodus* caught at Barrackpore, one, no. 216, was seen to be restless and ' fierce.' It would hop continuously during the day from one side of the cage to the other, or would remain for short periods grinning and snarling at the observer. On May 1st there was diarrhoea (?incontinence of faeces). Up to the 3rd this ' furious ' state persisted, and though the bat was in solitary confinement the fore limbs and forehead were seen to be lacerated from its attempts to escape through the wire of the cage. In the morning of the 4th it seemed, however, less excited, and on the following day it was normal and quiet. On that same day, April 5th, it was caged with a calf. The calf was bitten on the nose during the day of the 5th ; there was no biting on the 5th–6th or the 6th–7th ; on the 7th–8th he was bitten on the hind leg ; on the 8th–9th on the back ; on the 9th–10th on the same spot on the back ; and on the 10th–11th again on the back. On the morning of the 11th the calf was removed for observation. There was a gradual loss of flesh, and on June 2nd there was paralysis of the left side of the body. Death took place the following day, i.e., in 8 weeks. The hippocampus showed no Negri bodies. On June 8th brain material from this calf was injected intra-ocularly into rabbit 144. Death with paralysis took place in 24 days, and the hippocampus showed Negri bodies. On the 10th, another two-weeks-old calf was stalled with bat 216. During the night of the 10th–11th it was bitten on the back and left flank ; on the 12th–13th on the neck, back and left side ; on the 16th–17th on the left hip ; on the 17th–18th on the back and right ear ; on the 18th–19th on the neck ; on the 19th–20th on the left side of the abdomen ; and on the 21st–22nd on the shoulder and right flank. Biting did not take place during the 11th–12th, 13th–14th, 14th–15th, 15th–16th or 20th–21st. On the morning of the 22nd the calf was removed for observation. It has increased in size and has remained well up to the date of writing, July 31st, 1936.

4. On December 24th, 1935, six *Desmodus* bats, captured at Point Gourde on the 20th, were caged with a calf from Tobago. The calf was bitten on the 25th–26th on the left shoulder, and on the 26th–27th again on the left shoulder and on the left side of the neck. On the morning of the 27th the calf was removed. On January 9th, 1936, the calf was again caged with these same *Desmodus*. He was bitten on the 9th–10th on the left hip, neck and shoulder, and on the 10th–11th on the left leg, back, neck and ear. On the morning of the 11th the calf was removed. He remained alive and well—though he was obviously losing flesh—until April 12th, i.e., 3 months and 18 days after being first bitten, when paralysis appeared on the right side of the body ; death took place the following day. The hippocampus showed Negri bodies.

CLINICAL MANIFESTATIONS OF RABIES IN THE VAMPIRE

After artificial inoculation the virus of rabies affects vampire bats in a manner similar to other mammals. In the classical form of the disease in the vampire there is the usual incubation period, in many instances a prodromal or invasive stage, then a stage of excitement, and lastly one of paralysis, followed by death.

The incubation period varies considerably, as shown in the following Table. In experiment no. 2, in which there is evidence that the bats were not infected prior to inoculation, the minimum period was 9 days and the maximum 38, with an average of 25 days. The longest incubation period was 171 days, and the shortest 7, though in experiment no. 6 a possible development of symptoms may have taken place in 3 days. During this period the bat shows no abnormality, though unfortunately temperatures were not taken.

TABLE

Incubation period in days

Experiment 1			Experiment 2			Experiment 3			Experiment 4		
Bat		Days	Bat		Days	Inoculated February 7th, 1936			Inoculated February 28th, 1936		
1	=	—	3	=	9						
2	=	11	4	=	19	Bat		Days	Bat		Days
5	=	73	6	=	25						
9	=	73	7	=	38	1	=	over 150	1	=	14
11	=	171	8	=	35	2	=	,,	2	=	over 150
12	=	106	10	=	24	3	=	,,	3	=	,,
						4	=	,,	4	=	,,
Average		86	Average		25						

Experiment 5			Experiment 6			Experiment 7			Experiment 8		
Inoculated March 6th, 1936			Inoculated April 4th, 1936			Inoculated April 15th, 1936			Inoculated April 15th, 1936		
Bat		Days	Bat		Days	Bat		Days	Bat		Days
A	=	11	A	=	7 (?3)	1	=	over 105	1	=	over 105
B	=	12	B	=	over 116	2	=	,,	2	=	,,
C	=	12	C	=	,,	3	=	,,	3	=	,,
D	=	over 120	D	=	,,						

A prodromal stage of short duration, 12–24 hours, is sometimes seen; the bat is restless and easily irritated and there are muscular tremors, or it is apathetic and off its feed. This stage is rapidly followed by that of excitement and ' fury,' well exemplified by bat 10 of experiment 2, bat 1 of experiment 4,

and bat 9916 of experiment 2 of those caught in natural surroundings. During this period, the excitement and restlessness are increased. The normal bat hangs head downwards with its companions during the day; if disturbed, it will fly about for a short time, seeking to conceal itself, and will then settle down, giving the impression of wishing to be left alone. On the other hand, the bat infected with rabies, during the stage of excitement, will dart violently from one side of the cage to the other, or dash against the wire mesh towards the observer, continuing these actions for a prolonged period. When he settles down again his eyes will remain fixed upon the onlooker, and he will snarl and hiss, giving the impression that he is on the defensive or is ready to attack at the slightest provocation. He is *en garde*. Again, if a stick is inserted gently against a normal bat in a cage, he will retreat from it and seek some other position from which to hang; he is shy and retiring. A rabid bat in the stage of 'fury,' on the contrary, snaps immediately at the stick and gnaws at it, or darts at the wire straight towards the intruder. There is no shyness and no attempt to avoid a conflict, but aggressiveness and hostility at being disturbed. The stage of 'fury' lasts from one day to as long as 5 days, as in the case of bat 1 of experiment no. 4, and is followed either by a return to normal habits and health without paralysis or death, as in the case of bat 1 of experiment no. 4, or, what was usual, by a development of paralysis.

The paralysis may appear at first only as a paresis of one or other limb, or may develop suddenly as a 'stony' paralysis. It most commonly involves the wing muscles, or the muscles of one or other limb, but the muscles of the eyelids, of the neck and of the lower jaw have also been affected. When the jaw is affected, a typical picture is seen. Incontinence of urine from paralysis of the bladder was frequently observed, and in one instance there was a suspicion also of incontinence of faeces. The paralysis can be described as spreading, though at times one limb will be involved suddenly and completely, and then another. In the terminal stage the bat may be seen lying helpless on the floor of the cage, either on one side or forward on its chest and neck, or on its back, making vain efforts to move when disturbed. At times, even though paralysed and helpless, it will still attempt to snap and bite. In five instances the duration of the paralysis was one day, in two, two days, and in two others, four days. In its main features the paralysis suggested the disease as observed in man and animals, though it should here be emphasized that, while the strain of virus employed in this study was a human strain which had produced the paralytic form of rabies, its inoculation subcutaneously into vampires produced both the furious and the paralytic types of disease.

It will now readily be seen by examination of the experiments here detailed that rabies in the vampire bat may manifest itself in more than one form, as follows:—

1. The typical classical furious rabies, in which 'fury' is a prominent and predominating feature and is followed by a stage of paralysis and death.

2. The typical paralytic form of rabies, in which no stage of ' fury ' or excitement can be observed, but in which the paralysis runs on directly to death.

3. A furious form of rabies, in which the stage of ' fury ' is followed by recovery.

4. A furious form, in which the stage of ' fury ' runs on directly to death, without any intervening paralysis.

5. A form in which the bat is well, but dies suddenly and unexpectedly, without any previous evidence of illness.

6. A subclinical, or latent, form of infection, in which the bat continues to live without any apparent departure from normal. This form demands careful study.

It should, moreover, be added that when once paralysis appears in the first or second form of the disease there is no recovery ; further, in certain instances, one or more symptoms may have occurred during the night and been passed unnoticed.

LATENCY OF INFECTION

The entrance of a pathogenic organism or virus into an animal host may evoke a manifest, an abortive, or a latent form of disease. To the clinician the typical open and the abortive forms of infection are of greater importance and frequency, but to the sanitarian and public health officer the latent form is of equal, if not of the greatest, significance. The mental concepts involved in the first two terms are definite and clear, but there is some obscurity and confusion in the interpretation of the meaning of ' latency of infection.' Charles Nicolle (1935) distinguishes very sharply between a latent and a symptomless or inapparent infection. In the latter there is no clinical or anatomical sign of disease, though the tissues of the host continue to harbour the infective agent, while in the former there is a subacute or chronic state of disease without evident symptoms. Fortner (1936) describes an infection as inapparent or ' " silencieuse " quand il n'y a eu aucun signe de maladie,' and as ' " latente " quand il y a eu antérieurement infection manifeste.' In the production of infection, the virus of rabies in the vampire bats of Trinidad resembles that of psittacosis in parrots. Thus, Meyer and Eddie (1934), in an examination of 104 aviaries in California, containing 1,850 apparently healthy shell parrakeets, found that more than half of the aviaries were harbouring infected birds, though frank outbreaks of the disease were rare. The existence of such latent infections was discoverable only by post-mortem examination. Burnet (1935), out of 274 parrots and other birds, apparently healthy, examined in Australia, found 50 infected and 33 showing enlarged spleens from past infection with the virus of psittacosis. These birds were indigenous to Australia and were both wild and domestic. It is, however, remarkable that no case of human psittacosis has been known to occur in Australia in human beings or in parrots prior to 1934. Burnet is of the opinion that this state of infection of parrots in Australia

has existed for centuries, and that the usual form of infection is a symptomless or latent one. Psittacosis, he says, must be regarded ' as an almost universally present low-grade infection of parrots [not only in Australia but in South America] which only on rare occasions flares up into the dramatically infective human disease which characterised the outbreaks of 1929 and 1930.'

In Trinidad, the development of paralytic symptoms in rabbits after intracerebral inoculation of material from bat brains, which show no Negri bodies, raised at first some suspicion as to the possibility of some unknown virus being present in bats, which was responsible for the symptoms in rabbits ; but further investigation revealed that a high proportion of apparently healthy bats without any obvious symptoms of disease was definitely infected with rabies. The virus in bats is, therefore, of low virulence but of high infectivity. Pawan (1936) reported a *Desmodus* bat, no. 4290—which had lived in captivity for $5\frac{1}{2}$ months—to be harbouring the virus of rabies in its brain, without any evidence of disease or abnormality in its habit. In the experiments here recorded, it is seen that the virus may produce furious rabies in vampire bats, from which there can be complete recovery, the bats remaining normal and apparently healthy, though still capable of transmitting rabies to susceptible animals. Such a manifestation of infection falls into the category of typical latent infection in Nicolle's sense. But, in addition, there are instances where even repeated lethal infections with the virus failed to elicit any abnormal symptom or evidence of disease, thus grouping this form of infection as a symptomless, silent or inapparent one, though, in order to conform to the strict definition, histological examination for any anatomical departure from the normal would be necessary.

DISCUSSION

The dog, or rather the canine tribe, is the genuine specific host of the virus of rabies. This close association was known to the ancient Egyptians, and, as pointed out by Kraus, Gerlach, and Schweinburg (1926), is referred to by Homer, Xenophon, Aristotle, Plutarch and others. Its appearance in the vampire bats in South Brazil in 1908, and in Trinidad in 1925 as an epizootic, must therefore be regarded as a form of aberrant or strayed parasitism, in Theobald Smith's (1934) sense. Such a migration of a parasite to a new host often leads to an alteration in the biological properties of the parasite and the formation of a new variant or mutant. ' Aberrancy,' says Smith, ' is the adventurous element in the life of a parasite which leads either to death or to new conquest.' Unfortunately, this new variant, which is capable of producing only a parasitic form of disease in man and domestic animals in Trinidad, found in the bat not only a suitable medium for survival, but a ready means of dissemination, through the necessity imposed upon the vampire of having to inflict a wound with its teeth in order to obtain blood as its food. The gregarious habits of bats, and the biting propensities of the *Desmodus*, readily

ensure propagation and spread of infection amongst them. But, in addition, an unusual biological process—unusual in reference to rabies—has also come into operation. In their mode of response to invasion by the virus of rabies, vampire bats do not all react similarly. Some develop the disease in its classical form, with death supervening in the allotted time; others show evidence of a mild or latent infection, with recovery and the development of a carrier state; while others again are refractory to infection and show no clinical evidence of disease, though capable of transmitting infection. This refractoriness can only be explained as due in some instances to resistance acquired endemically after birth, following recovery from disease, and in others to a natural or genetic immunity. Cases of alleged recovery from rabies in man have been recorded, but the consensus of opinion suggests that these were instances of ' lyssaphobia ' or hysterical manifestations. Joseph Koch (1930) is insistent on the existence of abortive rabies and of recovery from rabies, particularly in laboratory animals under experimental conditions, and quotes instances from the days of Pasteur onwards; but the results here recorded—in connection with the vampire bat—of recovery from rabies, and the development of the carrier state with ability to infect, are definitely unusual and contrary to previous experience. They are almost heretical.

The first impact of a pathogenic organism or virus on the population of a new susceptible host is frequently the production of a virulent or fatal disease, with the destruction and elimination of all but the naturally resistant. In the early period of the outbreak of rabies in Trinidad in 1926–31, authentic accounts were given of bats fighting with one another during the day and of attacking man and animals in bright sunshine. Such phenomena suggested the typical furious type of rabies, appearing as a virulent disease attacking a non-immune, unprotected population. Recently, however, from 1933 onwards, such reports have not been received, pointing to the survival of only the naturally immune and to the development of mild subclinical infections, together with the establishment of a balanced equilibrium between the bat host and the rabies virus-parasite, as is well exemplified in parrots and psittacosis in Australia. If this theory is correct, the disease was not indigenous to the bat fauna of Trinidad, and the virus gained access to vampires for the first time about the year 1925.

Early in this investigation it was discovered that the virus of rabies after subcutaneous inoculation may appear in the brains of vampires as early as 9 days after infection, without evoking any abnormal manifestations, and may remain there, viable and virulent, for prolonged periods. This phenomenon bore some resemblance to what Remlinger and Bailly (1932) describe as ' le phénomène de la tortue.' These workers found that the virus of rabies, if injected into the brains of land and fresh-water turtles, becomes localized there, and remains virulent for as long as 10 months. The spinal cord of such turtles do not, however, show infection, which is therefore an inapparent and passive one—' une conservation banale.' In ' Oulo-fato,' a form of rabies in indigenous

dogs in French West Africa, Nicolau, Mathis and Constantinesco (1933) explain the comparative rarity of the transmission of infection to man by the fact that the virus shows little tendency to pass to the salivary glands, and that the saliva, as judged by experimental study on rabbits, is not infectious. In the case of the vampire bat, the virus spreads after subcutaneous inoculation not only to the brain, but also to the salivary glands and externally through the saliva, which may be highly virulent as early as 7 days after infection, while the bat itself may manifest no sign of disease.

The strain of virus employed in this study was a human strain which had, of course, produced paralytic rabies, but its inoculation into vampire bats led in certain instances to the development of furious rabies. Such bats, with furious rabies, after biting calves infected them with rabies, but again of the paralytic type. The virus, while capable of producing the furious form of the disease in vampires, after passage through them, is so modified as again to produce the paralytic disease. There is reason for believing that a modification in the biological property of the virus did actually occur under natural conditions in Trinidad. In Brazil up to the present, and in Trinidad from 1925 to 1929, only certain animals were affected, no case being reported for man or dog. Suddenly, however, in 1929, in the inland village of Siparia, in Trinidad, 13 human beings were stricken and died. From 1925, when the disease first appeared in animals throughout the island, to 1929, when the first human case was reported, vampire bats were biting human beings, and it cannot be proved that they were bitten only by non-infected ones. This highly probable modification or variation in the biological property of the virus supports the contention of Greenwood, Topley and their associates (1936), based on their work on experimental epidemiology in mice, that ' variations in the character of a bacterial parasite may well be of decisive importance in the secular history of any epidemic disease.'

The clinical manifestations in calves bitten by infected bats and kept under artificial conditions resemble those seen in calves injected intracerebrally with *virus fixe* of other rabies virus, and not the disease which occurs in nature from the bite of bats in the field, as described by Hurst and Pawan (1931) ; it may be that artificial confinement modifies the clinical course. The incubation period in days of ten calves bitten under experimental conditions was as follows : 150, 165, 159, 120, 97, 23, 20, 40, 28 and 108. The shortest incubation period was 20 days and the longest 165, with an average of 91 days, or roughly three months.

In a tropical country such as Trinidad, where live-stock is pastured in the open during both the day and the night, the opportunities for the transmission of infection by the bite of the vampire are great, while the poor labouring classes, living in proximity to wooded areas, and often in defective and imperfectly protected houses, are also exposed to their attacks. Such intimacy between vampire and man and beast offers easy facilities for the spread of the

disease. The discovery, therefore, in supposedly non-infected areas of infected bats which show no abnormal manifestation should lead to the expectation of a larger number of cases of rabies, especially in animals; but consideration must be given to the important fact emphasized by Curasson (1936) that Babes states that only 5–10 per cent. of persons bitten by rabid dogs develop rabies, while Hogyes declares that 86·1 per cent. of persons bitten escape infection without treatment. To what extent and over what period an infected vampire transmits infection to other bats and to domestic animals will be the subject of a further inquiry in Trinidad; but the results of this study demonstrate the necessity for destroying all vampires, irrespective of any abnormal behaviour, and the dangers attendant upon failure to resort to prophylactic treatment of all persons bitten by bats, without regard to the district in which they were bitten.

SUMMARY

1. Vampire bats are susceptible to artificial inoculation with the virus of rabies.

2. The disease thus produced may be of the furious or the paralytic type, or there may be no clinical symptoms.

3. Vampire bats, caught in natural conditions, may be found to be unduly excited and ' furious,' or may appear healthy without evidence of disease. They may, however, in both cases be infected with rabies.

4. Vampire bats with symptoms of furious rabies, or without any evidence of disease, following natural or artificial infection, are capable of transmitting rabies to animals by their bites.

5. Vampire bats may become carriers of rabies after ' recovery ' from the furious form of the disease. In this state they may remain capable of spreading infection by their bites for prolonged periods.

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CORRIGENDUM

'The transmission of paralytic rabies in Trinidad by the vampire bat (*Desmodus rotundus murinus* Wagner, 1840),' by J. L. Pawan, vol. 30, no. 1, April, 1936.

Page 115, 5th line from bottom of page : ' In every instance but one (case no. 31, ' C.B.') ' should read ' In nearly every instance where inquiry was made.'

RESEARCHES ON BLACKWATER FEVER IN GREECE*

IV.—EXPERIMENTAL INVESTIGATIONS INTO THE EXISTENCE OF HAEMOLYTIC STRAINS OF MALARIA AND/OR OTHER SPECIFIC PARASITES IN BLACKWATER FEVER

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With the object of limiting the field of discussion concerning the existence of haemolytic strains of malaria and or other specific parasites associated with the genesis of blackwater fever, we have carried out a series of blood inoculations and mosquito feeds from cases of blackwater fever to general paralytics and other mental patients. In all, 58 cases of blackwater fever were used and 106 mental patients.

Our work has fallen into two categories :—

(*a*) *Blood inoculations*

Eight c.cm. to 15 c.cm. of blood was drawn from one of the superficial veins in the arm, into sterile Bayer venules containing 7–12 mg. of neutral potassium oxalate, and transferred immediately by intramuscular injection into cases of general paralysis.

In carrying out this work, we selected cases of blackwater fever after varying periods from the first passage of urine containing haemoglobin. Thus our bloods have been drawn from cases of haemoglobinuria from 3 days before the first passage of black urine to 25 days after. Further, we endeavoured to get bloods from our cases of haemoglobinuria as representative as possible with regard to their malaria-parasite content. The absence of quartan parasites from our cases will be shown in a later paper to have no significance so far as blackwater fever in Greece is concerned. It will be observed further that the bloods were chosen on different days, so as to include as far as possible all types of parasites for the various days. We thus have a fairly complete series of blood inoculations from a very representative group of blackwater fever cases.

In some instances, the blood taken from the case of blackwater was negative for malaria ; in other cases, it was positive for either *P. vivax* or

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P. falciparum, or both. In some cases the infection was a very heavy one (more than 10 parasites per thick film oil immersion field), and in others light (less than one parasite per thick film oil immersion field).

The general paralytic recipients were in some cases negative for malaria, and had been so for many months previous to our inoculation. In other cases they were suffering from either acute or chronic malaria. A few of the mental cases gave a history of never having had malaria.

In some of the cases the negative general paralytics became positive for malaria after varying periods of incubation. In many cases, where malaria-negative blood had been taken from our case of haemoglobinuria and injected into known negative general paralytics, malaria supervened, showing that blackwater fever blood that has been shown to be negative by several thick smear examinations is not necessarily devoid of parasites.

In other cases, where a known heavily positive blood has been used for the inoculation, no malaria has developed in the mental patient, showing refractoriness or other factors at work preventing the development of malaria.

In no instance did a case of blackwater fever develop as a result of these blood inoculations. The protocols for this blood work are given in Table I, column 1 of which denotes the number of days that had elapsed after the first passage of black urine when our blood was taken. The minus signs denote the days before the first passage of black urine, and the plus signs denote the days after. The letter that appears after the number in column 2 refers to blood taken from different patients after the lapse of the same period of time from the first passage of black urine. Thus, for the third day after the passage of urine containing haemoglobin there were three patients from whom blood was taken, denoted in column 2 by the letters A, B and C.

In the third column the parasite-content of each blood is given under the title of the same letters as appear in column 2.

In column 4, are given the same letters with a number affixed, thus : A 1, A 2 ; B 1, B 2 ; C 1, C 2. This indicates that the original bloods, A, B, C, were divided into two parts, and that a part was given to two separate mental patients, A 1 and A 2, etc.

In column 5, are given the results of the blood inoculation into the mental patients, under the same headings of A 1, A 2 ; B 1, B 2 ; etc. Thus, for the third day three blackwater fevers were available, A, B and C (as shown in column 2). The parasite-content of each of these bloods is shown in column 3. Each of these bloods was divided into two parts, as shown in column 4, and injected into six mental patients. The results of the injection is shown in column 5, where the negative blood from blackwater fever case A was divided into two parts and given to each of two mental patients. Blood was taken from these mental patients by thick smear after a lapse of 7, 15, 22 and 30 days from the inoculation. A positive found on any of these days is noted in column 5.

TABLE I

Blood inoculations

No. of days before (−) or after (+) 1st passage of black urine that blood was taken	No. of cases of B.W.F. from which blood was taken	Parasites present in B.W.F. blood	No. of cases of G.P.I. into which blood was injected	Results of injection into G.P.I. cases
— 3 days	A 2 B	A. *vivax* ano. B. *falcip.* ano.	A 2 B	A. *vivax* B. *falcip.*
— 2 „	0	0	0	0
— 1 „	1	*vivax* ano.	1	*vivax*
+ ½ „	A 2 B	A. *falcip.* gam. B. Neg.	A 1 A 2 4 B 1 B 2	A 1 – *falcip.* A 2 – *falcip.* B 1 – Neg. B 2 – Neg.
+ 1 „	A B 4 C D	A. Neg. B. Neg. C. *vivax* ano. D. *falcip.* ano.	A B 4 C D	A – *vivax* B – Neg. C – *vivax* D – *falcip.* + *vivax*
+ 2 „	A 2 B	A. *vivax* ano. B. *vivax* ano.	A 1 A 2 4 B 1 B 2	A 1 – *vivax* A 2 – Neg. B 1 – *vivax* B 2 – *vivax*
+ 3 „	A 3 B C	A. *falcip.* gam. B. Neg. C. *vivax* ano.	A 1 A 2 B 1 6 B 2 C 1 C 2	A 1 – *falcip.* A 2 – Neg. B 1 – *falcip.* ano. B 2 – *falcip.* ano. C 1 – *vivax* C 2 – Neg.
+ 4 „	1	Negative	A 2 B	A – *vivax* B – Neg.
+ 5 „	A 2 B	A. Neg. B. Neg.	A 1 A 2 4 B 1 B 2	A 1 – Neg. A 2 – Neg. B 1 – Neg. B 2 – Neg.

ano. = rings ; gam. = gametocytes.

TABLE I—(*Continued*)

Blood inoculations

No. of days before (−) or after (+) 1st passage of black urine that blood was taken	No. of cases of B.W.F. from which blood was taken	Parasites present in B.W.F. blood	No. of cases of G.P.I. into which blood was injected	Results of injection into G.P.I. cases
+ 6 days	A 3 B C	A. *vivax* amb. B. *vivax* gam. C. Neg.	A 1 A 2 B 1 6 B 2 C 1 C 2	A 1 – *vivax* A 2 – *vivax* B 1 – Neg. B 2 – Neg. C 1 – *vivax* C 2 – *vivax*
+ 7 „	0	0	0	0
+ 8 „	A 2 B	A. Neg. B. Neg.	A 1 A 2 4 B 1 B 2	A 1 – Neg. A 2 – Neg. B 1 – Neg. B 2 – Neg.
+ 9 „	A 3 B C	A. Neg. B. Neg. C. *falcip.* ano.	A 3 B C	A – Neg. B – *vivax* C – *falcip.*
+ 10 „	A 2 B	A. *falcip.* ano. B. *vivax* ano.	A 1 A 2 4 B 1 B 2	A 1 – *falcip.* A 2 – *vivax* + *falcip.* B 1 – *vivax* B 2 – Neg.
+ 11 „	A 2 B	A. *vivax* ano. B. Neg.	A 1 A 2 4 B 1 B 2	A 1 – *falcip.* + *vivax* A 2 – *vivax* B 1 – Neg. B 2 – Neg.
+ 12 „	A B 4 C D	A. Neg. B. *vivax* amb. C. *falcip.* ano. D. Neg.	A 1 A 2 B 1 B 2 8 C 1 C 2 D 1 D 2	A 1 – Neg. A 2 – Neg. B 1 – *vivax* B 2 – Neg. C 1 – *falcip.* C 2 – *falcip.* D 1 – Neg. D 2 – Neg.

ano. = rings ; amb. = amoebae ; gam. = gametocytes.

TABLE I—(*Continued*)

Blood inoculations

No. of days before (−) or after (+) 1st passage of black urine that blood was taken	No. of cases of B.W.F. from which blood was taken	Parasites present in B.W.F. blood	No. of cases of G.P.I. into which blood was injected	Results of injection into G.P.I. cases
+ 13 days	A	A. *falcip.* + *vivax*	A 1	A 1 – *falcip.*
			A 2	A 2 – *falcip.* + *vivax*
	B	B. Neg.	B 1	B 1 – Neg.
			B 2	B 2 – Neg.
	4		8	
	C	C. *vivax* amb.	C 1	C 1 – *vivax*
			C 2	C 2 – *vivax*
	D	D. Neg.	D 1	D 1 – Neg.
			D 2	D 2 – Neg.
+ 14 „	A	A. Neg.	A 1	A 1 – *falcip.*
			A 2	A 2 – *falcip.*
	2		4	
	B	B. Neg.	B 1	B 1 – Neg.
			B 2	B 2 – Neg.
+ 15 „	0	0	0	0
+ 16 „	A	A. *falcip.* gam.	A 1	A 1 – Neg.
			A 2	A 2 – Neg.
			B 1	B 1 – *vivax*
	3 B	B. *vivax* ano.	6 B 2	B 2 – *vivax*
	C	C. Neg.	C 1	C 1 – *vivax*
			C 2	C 2 – *vivax*
+ 17 „	A	A. *falcip.*	A 1	A 1 – *falcip.*
			A 2	A 2 – Neg.
	2		4	
	B	B. Neg.	B 1	B 1 – Neg.
			B 2	B 2 – Neg.
+ 18 „	A	A. *vivax* amb.	A 1	A 1 – *vivax*
			A 2	A 2 – *vivax*
			B 1	B 1 – *falcip.*
	B	B. *falcip.* ano.	B 2	B 2 – *falcip.*
	4		8	
	C	C. Neg.	C 1	C 1 – *vivax*
			C 2	C 2 – *vivax*
	D	D. Neg.	D 1	D 1 – Neg.
			D 2	D 2 – Neg.

ano. = rings ; amb. = amoebae ; gam. = gametocytes.

TABLE I—(*Continued*)

Blood inoculations

No. of days before (−) or after (+) 1st passage of black urine that blood was taken	No. of cases of B.W.F. from which blood was taken	Parasites present in B.W.F. blood	No. of cases of G.P.I. into which blood was injected	Results of injection into G.P.I. cases
+ 19 days	1	Negative	A 2 B	A – Neg. B – Neg.
+ 20 „	A 2 B	A. *vivax*+*falcip.*ano. B. Neg.	A 1 A 2 4 B 1 B 2	A 1 – *vivax* + *falcip.* A 2 – *vivax* B 1 – Neg. B 2 – Neg.
+ 21 „	1	*vivax* gam.	A 2 B	A – Neg. B – Neg.
+ 23 „	A 2 B	A. *falcip.*+*vivax* ano. B. Neg.	A 1 A 2 4 B 1 B 2	A 1 – *falcip.* A 2 – *falcip.* B 1 – *vivax* B 2 – Neg.
+ 25 „	1	Negative	A 2 B	A – Neg. B – Neg.
+ 27 „	A 2 B	A. *vivax* ano. B. Neg.	A 1 A 2 4 B 1 B 2	A 1 – *vivax* A 2 – Neg. B 1 – Neg. B 2 – Neg.
+ 30 „	1	*falcip.* + *vivax* ano.	A 2 B	A – *falcip.* + *vivax* B – *vivax*
Totals	58		106	

ano. = rings ; gam. = gametocytes.

(*b*) *Mosquito feeds*

The three vectors of malaria in Macedonia would appear, from the work of Barber and Rice (1935), to be *A. superpictus*, *A. elutus* and *A. maculipennis*. The most important of these is *elutus*, with *superpictus* falling some way behind; *maculipennis* would not seem to be an important vector in this part of Greece. The number of *elutus* that we had available for our work was lamentably small; but work is continuing with this species next season and will be reported separately.

Our original plan was to use sterile mosquitoes reared from eggs, but we afterwards decided that, unless some positive results were forthcoming from the feeding of the non-sterile ones, this was an unnecessary labour. Had a case of blackwater fever developed in any of our mental cases, we should have considered repeating the work with sterile anophelines. Our experiments were carried out in a transmission-free period of the year (December to April), although from our point of view this was of no particular importance, except in so far as it enabled us to say whether the malaria developing in a mental case was due to our blood inoculation or mosquito feed. The blackwater fever cases on which our mosquitoes were fed sometimes contained gametocytes of *vivax* or *falciparum*, and in some cases, after suitable periods of incubation, malaria was transmitted to the mental case.

As in the case of the blood inoculations, we took cases of blackwater fever after the lapse of varying periods of time from the first passage of urine containing haemoglobin, and our cases include patients from 3 days before the first passage of black urine up to 30 days after.

The work with the mosquitoes was rendered somewhat onerous on account of the fact that we felt it necessary to incubate the mosquitoes for varying periods after their feed on the blackwater fever case. We decided to do this at 26° C. to 30° C. for periods from 2 to 30 days after their blackwater fever feed. In some cases we succeeded in keeping our mosquitoes going for as long as 50 days after their first feed.

After having been fed on a case of blackwater fever, the mosquitoes were placed in an incubator and incubated for the decided period, and were then ' back fed ' on our mental patients.

From the figures given in Table II it will be seen that a mosquito that had been fed on a given case of blackwater fever might after different periods of incubation be ' back fed ' on several different mental patients. In Table II the protocols for the mosquito work are given in a somewhat condensed form. Column 1 denotes the number of days that had elapsed from the first passage of black urine, when our mosquitoes were fed; column 2, the number and species of mosquitoes fed; column 3, the days of incubation of the mosquitoes; column 4, the number of cases of blackwater fever available for the particular day, together with the parasite-content of the patients' blood; column 5, the number of mental cases on which the mosquitoes were fed; and column 6, the results

TABLE II

Mosquito feeds

No. of days from 1st passage of black urine on which mosquitoes were fed	No. and species of mosquitoes fed	No. of days mosquitoes were incubated after B.W.F. feed	No. of cases of B.W.F. on which mosquitoes fed and parasite-content of blood	No. of cases of G.P.I. on which mosquitoes were fed	Results of feeding mosquitoes on G.P.I.
— 3 days	12 *superpictus* 8 *maculipennis* 6 *elutus*	4, 6, 8 10, 12	A. *vivax* ano. 2 B. *vivax* amb.	A 1 A 2 4 B 1 B 2	A 1 – Neg. A 2 – Neg. B 1 – Neg. B 2 – Neg.
— 2 „	6 *superpictus* 4 *maculipennis* 0 *elutus*	2, 7, 9 15, 20, 24	1 Neg.	A 2 B	A – Neg. B – Neg.
+ ½ „	10 *superpictus* 8 *maculipennis* 4 *elutus*	3, 5, 7, 9, 11 13, 17, 22	A. *falcip.* ano. 2 B. Neg.	A 1 A 2 4 B 1 B 2	A 1 – Neg. A 2 – Neg. B 1 – Neg. B 2 – Neg.
+ 1 „	8 *superpictus* 4 *maculipennis* 2 *elutus*	12, 19, 25 27, 30, 35	A. Neg. 3 B. *vivax* amb. C. *falcip.* ano.	A 1 A 2 B 1 6 B 2 C 1 C 2	A 1 – Neg. A 2 – Neg. B 1 – Neg. B 2 – Neg. C 1 – Neg. C 2 – Neg.
+ 2 „	12 *superpictus* 6 *maculipennis* 6 *elutus*	3, 5, 7, 9, 11 12, 15, 19 22, 28	A. *vivax* amb. 2 B. *vivax* amb.	A 1 A 2 4 B 1 B 2	A 1 – Neg. A 2 – Neg. B 1 – Neg. B 2 – Neg.
+ 3 „	6 *superpictus* 6 *maculipennis* 6 *elutus*	12, 15, 18 20, 24, 27	A. *falcip.*+gam. 2 B. *vivax* ano.	A 1 A 2 4 B 1 B 2	A 1 – *falcip.* A 2 – *falcip.* B 1 – Neg. B 2 – Neg.
+ 5 „	8 *superpictus* 6 *maculipennis* 4 *elutus*	2, 4, 6, 8 10, 12, 16 20, 22, 24	A. Neg. 2 B. Neg.	A 1 A 2 4 B 1 B 2	A 1 – Neg. A 2 – Neg. B 1 – Neg. B 2 – Neg.

ano. = rings ; amb. = amoebae ; gam. = gametocytes.

TABLE II—(*Continued*)

Mosquito feeds

No. of days from 1st passage of black urine on which mosquitoes were fed	No. and species of mosquitoes fed	No. of days mosquitoes were incubated after B.W.F. feed	No. of cases of B.W.F. on which mosquitoes fed and parasite-content of blood	No. of cases of G.P.I. on which mosquitoes were fed	Results of feeding mosquitoes on G.P.I.
+ 6 days	12 *superpictus* 6 *maculipennis* 6 *elutus*	3, 5, 7, 9, 11 13, 15, 17, 20 23, 26, 30	A. *vivax* gam. 2 B. *vivax* ano.	A 1 A 2 4 B 1 B 2	A 1 – *vivax* A 2 – *vivax* B 1 – Neg. B 2 – Neg.
+ 8 „	6 *superpictus* 4 *maculipennis* 0 *elutus*	2, 4, 6, 8, 10 12, 16, 18, 20 23, 26, 30	A. Neg. 2 B. Neg.	A 2 B	A – Neg. B – Neg.
+ 9 „	4 *superpictus* 2 *maculipennis* 2 *elutus*	4, 8, 10, 12 14, 17, 21 25, 30, 35	1 *falcip.* ano.	A 2 B	A – Neg. B – Neg.
+ 10 „	8 *superpictus* 4 *maculipennis* 0 *elutus*	3, 5, 7, 9, 11 13, 15, 17, 20 23, 25, 30	A. *falcip.* ano. 2 B. *vivax* amb.	A 1 A 2 4 B 1 B 2	A 1 – Neg. A 2 – Neg. B 1 – Neg. B 2 – Neg.
+ 11 „	12 *superpictus* 8 *maculipennis* 6 *elutus*	2, 5, 8, 11, 13 17, 19, 22 25, 29, 32	1 *vivax* ano.	A 2 B	A – Neg. B – Neg.
+ 12 „	6 *superpictus* 6 *maculipennis* 3 *elutus*	3, 7, 9, 12 16, 19, 22 27, 35	A. *vivax* ano. 2 B. *falcip.* ano.	A 1 A 2 4 B 1 B 2	A 1 – Neg. A 2 – Neg. B 1 – Neg. B 2 – Neg.
+ 13 „	8 *superpictus* 4 *maculipennis* 2 *elutus*	2, 4, 7, 9 10, 13, 16 18, 20, 22	A. *vivax* + *fal.* 2 B. *vivax* amb.	A 1 A 2 4 B 1 B 2	A 1 – Neg. A 2 – Neg. B 1 – Neg. B 2 – Neg.
+ 16 „	12 *superpictus* 6 *maculipennis* 6 *elutus*	2, 4, 6, 8, 9 12, 14, 16, 18 20, 22, 26, 30	A. *falcip.* gam. 2 B. *vivax* ano.	A 1 A 2 4 B 1 B 2	A 1 – *falcip.* A 2 – *falcip.* + *vivax* B 1 – Neg. B 2 – Neg.

ano. = rings ; amb. = amoebae ; gam. = gametocytes.

TABLE II—(*Continued*)

Mosquito feeds

No. of days from 1st passage of black urine on which mosquitoes were fed	No. and species of mosquitoes fed	No. of days mosquitoes were incubated after B.W.F. feed	No. of cases of B.W.F. on which mosquitoes fed and parasite-content of blood	No. of cases of G.P.I. on which mosquitoes were fed	Results of feeding mosquitoes on G.P.I.
				A 1	A 1 – Neg.
	6 *superpictus*	3, 7, 9, 11	A. *vivax* ano.	A 2	A 2 – Neg.
+ 18 days	3 *maculipennis*	15, 17, 20	2	4	
	0 *elutus*	25, 27, 30	B. *falcip.* ano.	B 1	B 1 – Neg.
				B 2	B 2 – Neg.
	3 *superpictus*	2, 5, 8, 11		A	A – Neg.
+ 20 „	3 *maculipennis*	16, 18, 21	1 *vivax* + *falcip.*	2	
	3 *elutus*	24, 28, 32		B	B – Neg.
	8 *superpictus*	7, 9, 11, 13		A	A – *vivax*
+ 21 „	10 *maculipennis*	18, 20, 24	1 *vivax* gam.	2	
	4 *elutus*	27, 35		B	B – Neg.
	6 *superpictus*	2, 4, 7, 11		A	A – Neg.
+ 25 „	12 *maculipennis*	13, 18, 22	1 Negative	2	
	4 *elutus*	31		B	B – Neg.
	6 *superpictus*	3, 8, 12		A	A – Neg.
+ 27 „	6 *maculipennis*	17, 21, 24	1 *vivax* amb.	2	
	2 *elutus*	28, 33		B	B – Neg.
	11 *superpictus*	5, 11, 13		A	A – Neg.
+ 30 „	8 *maculipennis*	18, 21, 25	1 *falcip.* + *vivax* ano.	2	
	4 *elutus*	30		B	B – Neg.
		Totals	35	68	

ano. = rings ; amb. = amoebae ; gam. = gametocytes.

of these feeds. The letters with the attached figures in columns 4, 5 and 6 refer to the number of cases of blackwater fever available for each day, together with their parasite-content. Thus, for the + 3-day period the mosquitoes available were fed on two cases of blackwater fever (A and B), and after varying periods of incubation were ' back fed ' on four mental patients, denoted by A 1, A 2, B 1, B 2 in column 5, the results of the feeds being given in column 6. As in the case of the blood inoculations, the results were all negative.

SUMMARY AND CONCLUSIONS

The inoculation of malaria-positive and negative blood, taken, after varying periods of time from the first passage of black urine, from 58 cases of blackwater fever and injected into 106 mental patients, failed in every case to produce haemoglobinuria.

Anophelines of the species *elutus*, *superpictus* and *maculipennis*, fed on 35 different cases of blackwater fever, after the lapse of varying periods of time from the first passage of urine containing haemoglobin, and after different periods of incubation at 26° C. 'back fed' on 68 cases of general paralysis, never produced a case of blackwater fever. This work would seem to us to limit the field of argument for the existence of haemolytic strains of malaria and or other specific parasites concerned in the genesis of blackwater fever, as has been advocated from time to time by various authors. The blood inoculations were in many instances carried out on mental patients with a suitable 'malaria ground,' in that they were suffering either from acute malaria, with their bloods loaded with *vivax*, *falciparum*, quartan or mixed infections, or from chronic malaria with low-grade infections. The blood used in these instances was frequently heavily infected with either *vivax* or *falciparum*, or both.

All the cases were kept under observation for from 9 to 18 months after their blood inoculation or mosquito feed.

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THE PROBLEM OF SPECIES, WITH SPECIAL REFERENCE TO THE MALARIA PARASITES*

BY

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Manson-Bahr, the biographer of Sir Patrick Manson, once said that one of the most important results of this great scientist's work was ' reminding the medical profession that medical *science* is rooted and *grows* in biology ' (Manson-Bahr and Alcock, 1927); and so perhaps we may hold Manson indirectly responsible for the planning of this very interesting symposium, as well as of the others which make up the programme of this conference. In any event, our power to control malaria in this modern day is very largely due to our knowledge of the biology of the parasite, and this again we owe in large measure, though indirectly, to Manson.

I wish to discuss briefly the biology of the malaria parasites as it relates to the problem of species, a problem which seems to me of increasing importance, and one which recent work on the malaria parasites of birds has brought very much to the fore. This is illustrated by a remark of Brumpt (1935) in a recent paper, that there are at least a dozen species of malaria in birds alone, and it may be added that the total number which have been described in birds is at least double this. It is obvious that many of these are not valid, and might not have been described at all had there been a clearer conception of what a species of malaria parasite is, and a better understanding of the tests of specificity. The question which I wish to ask is: ' How shall we conceive of and how shall we define a species of malaria plasmodium ? What tests shall we apply to discover whether a given species is valid? '

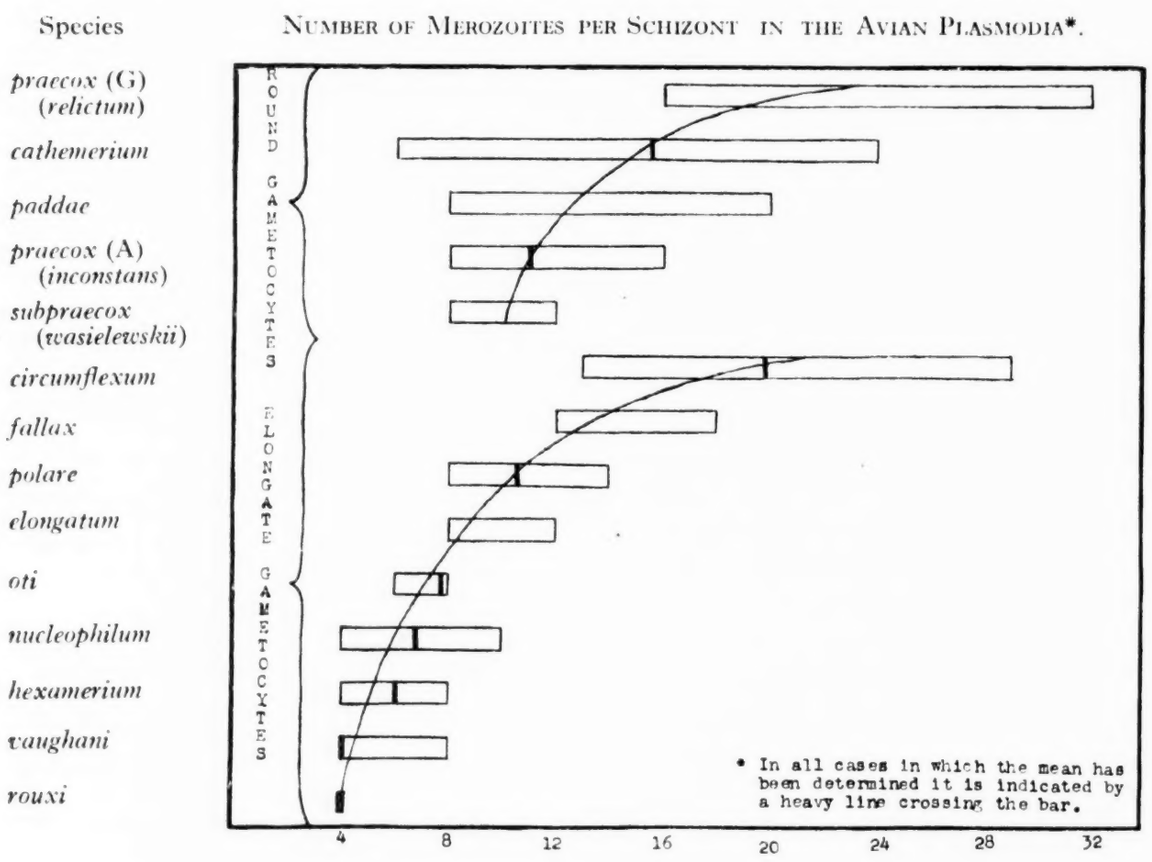
There are various definitions of species, but it is usually considered that the members of a species must share certain morphological characteristics, more or less peculiar to themselves, and that crossing with other such groups should not occur, though there are, of course, some exceptions to this rule. When dealing with parasites, certain other facts depending on the relationship between the host and parasite are also of use.

We find, however, that in the case of the malaria parasites there is little agreement as to the proper criteria of species, for certain species are accepted as valid by some and rejected by others. Morphological characteristics, of course, come first as a means of classification. Other types of characters which have

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been used include host-parasite specificity, effect of the parasite on the erythrocyte, length of asexual cycle, pathogenicity, reaction to anti-malarial drugs, climatic conditions controlling development in the mosquito, geographical distribution, types of cells parasitized (as in *P. elongatum*), etc.

But, as the number of species known increases, it becomes clear that none of these types of characters is by itself sufficient. We find, for example, that there is much overlapping in the numbers of merozoites produced, as the following figure shows :—



Similar difficulties are encountered when other morphological characters are used. The smaller species are so much alike, as far as the younger stages are concerned, that it is very difficult and often impossible to tell them apart. Differences in size do exist, but this is, of course, very variable. Pigment differs in colour, number of granules present in a given stage, shape of the individual granules, arrangement, and degree of refractility. Yet these peculiarities are also highly variable, even within a species, and are highly unreliable. Rather coarse, elongate granules may be seen on occasion in *P. praecox* (*relictum*), and relatively short and fine granules not infrequently in the gametocytes of *P. cathemerium*. Staining differences also exist, but they are not usually sufficient to identify species if considered alone. The shape of the gametocytes is,

of course, a very useful and quite constant character, but this only helps in determining into which of the two great groups of malaria parasites a given species falls, and it is becoming quite clear that the group characterized by elongate gametocytes is much the larger, in birds at least. It is my own opinion that these two groups should be recognized taxonomically, and I believe that the appropriate method of recognition would be the acceptance of two genera—the species producing round or irregular gametocytes being placed in the genus *Plasmodium* as at present, and those characterized by elongate or crescent-shaped gametocytes in the genus *Laverania* (Grassi and Feletti, 1890).

Host-parasite specificity has been regarded by many as a good test of species, but it is now quite clear that even in the human malarias this is not entirely rigid, as the recent work of Taliaferro (1934) shows. In birds it is notoriously slight, yet even here it is a very real thing. In nature, for example, it appears probable that *P. vaughani* occurs only in the robin, or perhaps in other birds of that family. Other species can be shown to be susceptible in the laboratory, however. Similarly, I have so far found *P. polare* only in the cliff swallow, although more than a thousand birds of somewhat more than fifty species have been examined. The canary is, however, susceptible to this species, just as it is to almost every other species with which it has been inoculated (although in this case the infection produced is relatively slight). And the chicken is apparently almost insusceptible to almost all the present-known species of avian malaria, though some of them will produce a very light and transitory infection in certain individuals (Manwell, 1933), and Brumpt (1935) has recently described under the name *P. gallinaceum* a strain of malaria observed in Indo-China by Broussais in 1910. Most of the species of avian malaria have been found in many species of hosts, however, and some of them, such as *capistrani* (see Russell, 1932), though naturally occurring in birds of other orders, have proved adaptable enough to infect the canary (a passerine bird) also. I have become convinced, as the result of careful study, that *capistrani* is indistinguishable from the stock American strain of *praecox*, and the figures of *P. gallinaceum* also show a very strong resemblance to this species. The very loose host-specificity of the avian malaria parasites is further illustrated by *P. circumflexum*, a very common species in passerine birds (see Manwell and Herman, 1935), and yet also occurring in the lesser yellow-legs (order Charadriiformes). It appears that host-specificity is also quite loose as far as the invertebrate host is concerned.

The effect of the parasite on the host-cell is also often regarded as an important specific character, and yet this may vary for a given species, or be virtually the same for a number of different species.

The types of cells parasitized in the vertebrate host may also be of value in defining a species, as Huff and Bloom (1935) have recently shown in the case of *P. elongatum*, which inhabits erythrocytes or erythroblasts by preference, but is not above living in other types of blood cells when convenient.

Periodicity is apparently another example of a variable character. Some

have thought that aestivo-autumnal malaria must be caused by two species of parasites, because the fever is in some cases quotidian and in others tertian ; but recent work has apparently shown that there are several strains of *P. praecox* among birds, each with a different periodicity; and it has also been demonstrated that the length of the asexual cycle in *P. cathemerium* depends on various environmental factors, such as the length of the day, time of feeding, etc. (Boyd, 1929, 1933). It may well be questioned, then, as to how valuable periodicity is as a measure of species. Pathogenicity is an even more variable character.

We also have other factors to contend with : for example, once the limits of variation within a species have been determined, how constant are they ? Can a species of malaria be shown to consist of races, as has been proved for various other parasitic and free-living forms ? There is increasing evidence that this may be true. If this is the case, where shall we stop in calling a group of malaria parasites a species, and begin speaking of it as a race or a variety ? There is also the much more fundamental question as to whether any of our ' species ' are in fact hybrids, or whether any of them can hybridize as the result of crossing within a mosquito, after the latter feeds on a mixed infection. Such infections are common in nature. It is even conceivable that if a given mosquito should chance to bite two different types of malaria-infected host in close succession, as, for example, a man and a bird, crossing of the malaria strains might occur even here. There is, to be sure, no experimental evidence for this, but it is a problem which should receive careful study, and it may be that the advice of Manson (1898) to Ross, though originally given in 1898, is still timely: ' Don't forget to work on avian blood plasmodia.' The avian malarias present particularly favourable material for research of this sort.

One thing more remains to be mentioned, and it relates to the immune reactions of the host, often called ' premunition ' in the case of malaria and some other highly chronic infections. In the past we have regarded this reaction as a highly specific thing—so specific that, if a host carrying a chronic infection of one species of malaria showed marked immunity against another strain, the two strains must, therefore, be of the same species. If, on the other hand, little or no protection existed, it was good evidence that the two strains involved were really distinct species. Some species of avian parasites have been differentiated largely on this basis, but the work of the Sergents (1931), Kikuth (1931) and Gingrich (1932) have shown that this again is a relative matter. My own work with some of the newly differentiated species (*nucleophilum*, *hexamerium*, *vaughani* and *rouxi*) has convinced me that, although these reactions are not very valuable as tests of specificity, they are, however, of some value as indications of zoological relationships, though they need to be interpreted in the light of morphological and other characters as well. But ' premunition ' which protects one individual against reinfection with the same or a different species may not do so in another, and different strains within a species may vary in this respect (see Sinton and Harbhagwan, 1935).

What is the conclusion then ? It seems to me that we should conceive of a species of malaria in genetic terms, as we do most other types of living things. It should be a strain of malaria plasmodium which will not hybridize with others in the invertebrate host, and it should, of course, have certain definite and more-or-less unique morphological characters. Until we know more about the behaviour of mixed infections in the mosquito, we shall, of course, have to continue to accept as species strains of malaria parasites which differ sufficiently morphologically, or otherwise, to be distinguished with reasonable certainty from others ; but we should recognize that some of them may in fact be only hybrids. We must also recognize the highly variable character of many of the customary attributes of malaria species. New ones should certainly not be accepted until they have been studied in the vertebrate host with some thoroughness.

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OBSERVATIONS ON *DIENTAMOEBA FRAGILIS*

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PREVALENCE OF *D. FRAGILIS*

In the text-books it is often stated that *D. fragilis* is a rare parasite ; many authors, in publishing the results of investigations on the frequency of intestinal protozoa, make no mention of it at all, and many others give a frequency of less than 1 per cent. On the other hand, in a fairly large number of papers the statement can be found that this amoeba occurs more often than is generally supposed. The discoverers (Jepps and Dobell, 1918) mention that it is probably more common than might be concluded from their figures. Thomson and Robertson (1929) and Wenrich, Stabler and Arnett (1935) make similar statements. And Nöller (1922), Brumpt (1927) and Doflein-Reichenow (1929) declare that it is not rare at all. In fact, some authors find *D. fragilis* one of the commoner, if not the most common of all, intestinal protozoa. Tochihara (1926) found it in 51 out of 124 persons, i.e., in 41 per cent., when six samples of each person were examined ; and Svensson (1928) in 11 out of 42 persons to whom a laxative had been administered, i.e., in 24 per cent.*

Of course, the frequency may be different in different countries, but this alone cannot explain the diversity of statements mentioned above. The technique of examination and the conditions under which the faecal samples are examined play a very important rôle—more so than with other intestinal protozoa. If most of the samples are solid, and are some hours old when examined, a frequent finding of *D. fragilis* could hardly be expected, even if a large portion of the individuals examined was infected with it. It is generally stated that *D. fragilis* can only be found in rather soft or fluid, fresh faecal material. Considering that these conditions often are not fulfilled, it is no wonder that this amoeba has often been missed.

In Holland, *D. fragilis* seems to be a common parasite. In 80 asylum inmates, to whom a saline laxative was given, I found it 29 times (36 per cent.) when examining one sample of each person. Cultural methods, as well as direct microscopical examination of fresh and stained films, were applied. As can be seen from Table I, *D. fragilis* was by far the most common intestinal protozoon. From this Table it is also evident that culture disclosed more otherwise undetected infections with this parasite than with any other.

* An extensive list of all the papers I could trace in which findings of *D. fragilis* have been reported has been published elsewhere (Brug, 1936*a*). It is too long to reproduce here.

TABLE I

Intestinal protozoa found in asylum inmates ; one sample of each, after a saline laxative

Species	No. of samples	Found by direct microscopical examination	Additional cases found by culture	Total percentages
Entamoeba histolytica	95	7 per cent.	1 per cent.	8 per cent.
Entamoeba tenuis (the 'small race' of *E. hist.*)	95	1 ,,	2 ,,	3 ,,
Entamoeba coli	95	13 ,,	2 ,,	15 ,,
Either *E. histolytica* or *E. coli* (trophozoites only)	95	3 ,,	9 ,,	12 ,,
Dientamoeba fragilis	80	12 ,,	24 ,,	36 ,,
Endolimax williamsi (*Jodamoeba bütschli*)	80	2·5 ,,	0	2·5 ,,
Endolimax nana	80	12 ,,	4 ,,	16 ,,
Trichomonas hominis	95	1 ,,	0	1 ,,
Chilomastix mesnili	95	2 ,,	0	2 ,,
Lamblia intestinalis	95	6 ,,	0	6 ,,

METHOD OF CULTURE

After many trials with samples known to contain intestinal protozoa, I came to the conclusion that the culture often failed because the fluid became acid in spite of the buffering action of solid and liquid horse serum. Results improved very much when I added to each culture-tube some milligrams of crystallized $CaCO_3$. Otherwise the medium was made after Dobell's prescription : horse serum solidified by heating, and as a fluid layer one part of horse serum and seven parts of Ringer-fluid and a little quantity of rice starch. The calcium carbonate has the drawback of counterbalancing in some unknown way the inhibitive action of rice starch on the growth of *Blastocystis*. The cultures often showed a parallel growth of *Blastocystis* and protozoa ; it seemed to me that luxurious *Blastocystis* growth did not usually crowd out the protozoa. To get rid of the *Blastocystis* one may try either to do without the calcium carbonate or to add 1 : 17,000 trypaflavine to the culture. However, these alterations sometimes kill the protozoa as well as the blastocysts.*

* For the influence of the addition of $CaCO_3$ to the medium on the growth of *Entamoeba histolytica* and other intestinal protozoa, see Brug (1936*b*).

THE MODE OF INFECTION OF MAN WITH *D. FRAGILIS*

Most intestinal protozoa are spread by means of cysts. *Trichomonas* does not produce cysts, but its trophozoite stage is rather resistant; apparently it can pass through the stomach without much injury. The spreading of *D. fragilis* can hardly be explained along these lines. It is said to die very soon after the stools are passed, and to occur in fluid or very soft stools only. Hence it would seem that infection with this parasite can only result from a concourse of special conditions rarely realized, and it would be quite intelligible if this infection were rare. However, in some groups of people infection is very common. Probably there exist many more such groups than are recorded in the literature. In view of this discordance it seemed desirable to retest the 'fragility' of *D. fragilis*.

HOW LONG CAN *D. FRAGILIS* SURVIVE IN STOOLS AFTER DEFAECATION?

During the investigation on the occurrence of protozoa in stools of inmates of an asylum, referred to above, I usually examined the material microscopically two hours after defaecation. I found that in 10 out of 29 found positive in cultures (inoculated with the absolutely fresh material) living *D. fragilis* could still be detected with ease. The diagnosis was in every case confirmed by stained slides. In four additional cases, small living amoebae, probably *D. fragilis*, were seen, but they were so few in number that I could not find them in the stained slides.

Having detected a carrier of *D. fragilis* whose stools I could examine as often as I wished, some experiments on the longevity of this amoeba were undertaken. In each experiment cultures were made in the medium described above, at various times after the passing of the stools. The results are shown in Table II.

From Table II it is apparent that in rather solid stools *D. fragilis* can survive at room-temperature for $6\frac{1}{2}$ hours, in semi-fluid stools for $13\frac{1}{2}$ hours. Attempts to cultivate the amoeba from stools 20 or more hours old did not succeed. The result of experiment 7 is somewhat unexpected: the culture of the fairly fresh dejection proved negative, that of stools 8–$13\frac{1}{2}$ hours old, positive. The supposition that some untoward incident had happened with the inoculation is disproved by the fact that the same thing happened again in the second stool produced after the same laxative.

D. FRAGILIS ALSO OCCURS IN 'NORMAL' STOOLS

The material that served for experiments 1–6 (Table II) appeared quite normal macroscopically. There was no question of the patient showing any signs of diarrhoea. But the portion of the faeces which was the last to be passed was somewhat softer than the rest, having been exposed during a shorter time to the desiccating action of the rectum. Inoculations were always made from

TABLE I

Intestinal protozoa found in asylum inmates ; one sample of each, after a saline laxative

Species	No. of samples	Found by direct microscopical examination	Additional cases found by culture	Total percentages
Entamoeba histolytica	95	7 per cent.	1 per cent.	8 per cent.
Entamoeba tenuis (the 'small race' of *E. hist.*)	95	1 „	2 „	3 „
Entamoeba coli	95	13 „	2 „	15 „
Either *E. histolytica* or *E. coli* (trophozoites only)	95	3 „	9 „	12 „
Dientamoeba fragilis	80	12 „	24 „	36 „
Endolimax williamsi (*Jodamoeba bütschli*)	80	2·5 „	0	2·5 „
Endolimax nana	80	12 „	4 „	16 „
Trichomonas hominis	95	1 „	0	1 „
Chilomastix mesnili	95	2 „	0	2 „
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TABLE II

Survival of *D. fragilis* in stools

Experiment no.	Nature of specimen	Saline laxative (+) or not (—)	Hours after defaecation	Result of culture	Temperature to which specimen was subjected before inoculation
1	Rather solid	—	3½ hours	Positive	Room-temperature
			4½ „	Negative	„ „
2	„	—	6 „	„	„ „
			6½ „	Positive	„ „
3	„	—	22½ „	Negative	Emulsified with tap-water and left at room-temperature
			22½ „	„	Same, and left at 28°
			22½ „	„	„ „ „ 37
4	„	—	8 „	„	Room-temperature
5	„	—	18 „	„	as in experiment 3
			18 „	„	„ „ 3
			18 „	„	„ „ 3
6	„	—	1½ „	„	room-temperature
			8 „	„	„ „
7	Semi-fluid	+	1¾ „	„	„ „
			8 „	Positive	„ „
			9 „	„	„ „
			10½ „	„	„ „
			11½ „	„	„ „
			12½ „	„	„ „
			13½ „	„	„ „
7a	Semi-fluid (second motion after same laxative)	+	¼ „	Negative	„ „
			7½ „	Positive	„ „
			9 „	„	„ „
			10 „	„	„ „
			11 „	„	„ „
			12 „	„	„ „
8	Pulpy	+	2 „	Negative	37
			2 „	„	28
			2 „	„	room-temperature
			25 „	„	37°
			25 „	„	28
			25 „	„	room-temperature
9	Frothy	—	0 „	Positive	„ „
			26 „	Negative	„ „
10	Semi-fluid	+	0 „	Positive	„ „
			24 „	Negative	„ „
			25, 27, 29 h.	„	„ „
11	„	+	1¼ „	Positive	„ „
			25¼ „	Negative	„ „

this portion. Direct microscopical examination usually showed *D. fragilis* to be present there ; only on rare occasions could it not be found.

These two facts—(*a*) that *D. fragilis* can survive 6½ hours in normal and 13½ hours in fluid stools, and (*b*) that a carrier regularly discharges it living—render the mode of infection a little less mysterious. However, they do not offer a satisfactory explanation for the frequent occurrence of the amoeba. Jepps and Dobell (1918) mention the possibility that *D. fragilis* is a natural parasite of some animal, producing cysts in that host, but not in man. So far, the amoeba has been found only in monkeys (Hegner), but no cysts were observed on that occasion.

IS *D. FRAGILIS* A FREE-LIVING AMOEBA?

Another possibility occurred to me, namely, that *D. fragilis* is a free-living amoeba, with the capacity to multiply in the human bowel, but producing cysts in the free-living state only. If so, it might be expected that the amoeba, being so easily cultivated, would grow well in cultures at room-temperature. Experiments to test this gave a negative result :—

Experiment 1.—From a tube containing 2,200 amoebae per c.mm., 6 tubes were inoculated ; tubes no. 1, 2 and 3 were left at 37° C. ; tubes no. 4, 5 and 6 at room-temperature. The result was :

Tube	1	2	3	4	5	6	
at outset ...	550	220	220	220	220	220	amoebae per c.mm.
after 1 day...	51	6	10	0	0	0	" "
" 2 days	740	6	4	0	0	0	" "
" 3 "	1,420	24	128	0	0	0	" "
" 4 "	520	97	574	0	0	0	" "
" 5 "	—	284	432	0	0	0	" "
" 7 "	—	452	48	0	0	0	" "
" 8 "	—	94	4	0	0	0	" "

Counts were made by shaking the tube vigorously until the contents looked homogeneous macroscopically, and by putting one drop into a self-made counting-chamber of 1 c.mm. Homogeneousness can be obtained more readily if the solid part of the medium is not sloping. At the end of the experiment, the deposit at the top of the solid part was examined likewise, in order to increase the probability that all the amoebae had actually disappeared in those tubes where, in 1 c.mm., nothing was found.

The result of this experiment was not quite conclusive, because the tubes at 37° were kept in an incubator in the dark, whereas the tubes at 20° were exposed to daylight. In another experiment both sets of tubes were kept in the dark. The results were as follows :—

Experiment 2.—From a tube containing 815 amoebae per c.mm., 5 tubes were inoculated. Tubes 1 and 2 were put into the incubator (37°) ; tubes 3, 4 and 5 were left at room-temperature (about 20°) in a closed tin. Result :

Tube	1	2	3	4	5	
at outset ...	163	163	163	163	163	amoebae per c.mm.
after 1 day...	50	72	0	0	0	" "
" 2 days	14	48	0	0	0	" "
" 3 "	58	700	0	0	0	" "
" 4 "	410	1,650	0	0	0	" "
" 5 "	700	—	0	0	0	" "
" 7 "	705	—	0	0	0	" "

deposit negative

— = no count.

Apparently in a culture maintained at room-temperature (about 20°) from the beginning onwards, not only is there no growth, but the amoebae are killed altogether. A closer inspection of the results of experiments 1 and 2 shows that transplantation to a fresh medium adversely affects the amoebae maintained at 37°. During the first day the amoebae in all the tubes suffered an important decrease in number. It may be even more than four days before the amoebae recover their initial numbers (experiment 1, tube 2). It seems quite possible that transplantation to a fresh medium has a similar effect in the tubes subsequently maintained at 20°, and that the death of the amoebae in these tubes is not due to cooling only, but to the combined effect of cooling and transplantation. Two further experiments confirmed this view :—

Experiment 3.—After vigorous shaking, the liquid portion of a three-days-old culture is divided into two equal parts, and each part is poured into a tube with fresh solid medium One of the tubes is kept at 37°, the other at about 20°. Result :

Amoebae per c.mm.	at outset	after 1 day	after 2 days
37° tube	810	646	632
20° „	810	255	87

Experiment 4.—The same experiment with a four-days-old culture gave a similar result :

Amoebae per c.mm.	at outset	after 1 day	after 2 days
37° tube	547	150	153
20° „	547	146	10

Under these conditions, therefore, the amoebae exposed for two days at room-temperature decrease in greater number than those kept at 37°. In subcultures started from the tubes exposed for 2 days to 20° there was a very good growth.

Another experiment, in which the influence of one day's exposure to room-temperature on cultures of different ages was tested, resulted as follows :—

Experiment 5.—From a three-days' culture containing 850 amoebae per c.mm., 4 tubes were inoculated with 182 amoebae per c.mm. Tube 1 was left at room-temperature during the first day and was then transferred to the incubator. Tube 2 was exposed to room-temperature during the second day only, tube 3 during the third day only, and tube 4 during the fourth day only. Result :

Tube	1	2	3	4	
at outset	*182	182	182	182	amoebae per c.mm.
after 1 day ...	* 0	*115	184	67	„ „
„ 2 days ...	0	* 0	*952	840	„ „
„ 3 „ ...	0	1	* 94	*1,394	„ „
„ 4 „ ...	0	7	64	* 374	„ „
„ 6 „ ...	0	1,122	711	731	„ „
„ 7 „ ...	—	629	653	575	„ „
„ 8 „ ...	0	—	—	—	„ „
„ 10 „ ...	0	—	—	—	„ „

* Exposure to room-temperature during 24 hours.
— = no count.

Apparently the noxious influence of cooling decreases with the age of the culture.

The effect on *D. fragilis* of a constant temperature of 28° C. is less deleterious than that of one of 20° :—

Experiment 6.—From a culture with 1,856 amoebae per c.mm., 6 tubes are inoculated, so that each tube started with 206 amoebae per c.mm. Tubes 1, 2 and 3 were put into the incubator ; tubes 4, 5 and 6 into the tropical room of the Institute, with a constant temperature of 28°C. Result :

Tube	1	2	3	4	5	6	
at outset ...	206	206	206	206	206	206	amoebae per c.mm.
after 1 day...	30	36	56	6	8	24	,, ,,
,, 2 days	16	272	398	2	6	8	,, ,,
,, 3 ,,	1	385	640	1	3	8	,, ,,
,, 4 ,,	192	705	780	0	0	3	,, ,,
,, 5 ,,	685	660	760	0	tube	1	,, ,,
,, 7 ,,	505	530	480	0	broken	0	,, ,,
,, 9 ,,	160	152	170	0		0	,, ,,

The amoebae found in tubes 4, 5 and 6 after one day and later were mostly of the degenerated, blastocystis-like type, with a large vacuole occupying almost the whole animal, as described by Jepps and Dobell (1918).

Although all these experiments do not disprove with absolute certainty that *D. fragilis* is a free-living amoeba, only accidentally parasitizing man, they are far more in favour of the old thesis that this amoeba is a true parasite.

THE LAG-PHASE IN CULTURES OF *D. FRAGILIS*

In the previous paragraph, attention was drawn to the fact that the cultures showed a decrease in the number of the amoebae during the first day at least, and usually for a longer period. There is certainly a lag-phase, therefore, which may be a prolonged one. In tube 2 of experiment 1, and in tube 1 of experiment 2, it was more than 4 and 3 days respectively before the initial number of amoebae was reached again. The loss of amoebae rarely fails to show during the first day, but if it does the increase in number is insignificant during that day as compared with that during the second day. In many cultures not mentioned here daily counts of the numbers of amoebae were made. In none of them was the lag-phase missing. An extremely heavy lag-phase was seen in the following case : no. of amoebae per c.mm. at outset 200 ; after 1 day 0 ; after 2 days 0 ; after 3 days 0 ; after 5 days 170 ; after 7 days 805 ; after 8 days 588.

As may be seen from the experiments mentioned in the previous paragraph, the lag-phase was rather variable in duration and intensity. From these and from other experiments not recorded here, I received the impression that the size of the inoculum had a decisive influence on the duration of the lag-phase. Experiments were planned to test this more systematically.

INFLUENCE OF THE SIZE OF THE INOCULUM UPON THE FATE OF THE CULTURE

Experiment 7.—From a culture containing 1,952 amoebae per c.mm., 3 tubes were inoculated with 97 amoebae per c.mm., and, by further dilution with fresh serum-Ringer, 3 tubes with 7 amoebae per c.mm.

Tube	1	2	3	4	5	6	
at outset ...	97	97	97	7	7	7	amoebae per c.mm.
after 1 day...	0	2	0	0	0	0	" "
" 2 days	10	20	2	0	0	0	" "
" 3 "	480	1,016	192	0	0	0	" "
" 5 "	434	40	526	0	0	0	" "
" 6 "	—	14	86	0	0	0	" "
" 7 "	—	22	136	0	0	0	" "
" 9 "	—	—	—	0	0	0	" "

— = no count.

Experiment 8.—Another experiment yielded a similar result :

Tube	...	1	2	3	4	5	
at outset ...	...	100	100	4	4	4	amoebae per c mm.
after 1 day...	...	56	90	0	0	0	" "
" 2 days	...	274	226	0	0	0	" "
" 3 "	...	550	496	—	0	0	" "
" 4 "	...	726	104	0	0	0	" "

— = no count.

Similar experiments repeated many times invariably resulted in the death of the cultures which had been started with a small inoculum. This seemed rather puzzling. Can it be explained by the assumption that part of the inoculated amoebae are unfit for multiplication, that in the slightly inoculated tubes none but unfit amoebae were introduced, and that in the tubes with a more bulky inoculum there were some healthy ones also ? The regularity with which the phenomenon occurred does not favour such an explanation. Moreover, the absolute number of amoebae present in the ' small ' inocula is not so very small. Each tube contained 4 or 5 c.cm. of diluted serum ; if inoculated with 4 amoebae per c.mm., the culture is started with about 16,000–20,000 amoebae, as against 400,000–500,000 amoebae in the tubes with 100 amoebae per c.mm. These figures are large enough to exclude what we might call bad chance, and to justify the supposition that the average ' quality ' of the amoebae in the small inoculum was the same as that in the large inoculum. It must be borne in mind, however, that the large inoculum not only contains more amoebae, but also more ' old medium,' i.e., more old serum, more bacteria, more products of metabolism of bacteria and amoebae. It is possible that the amoebae in the scantily inoculated tubes did not grow—and even died—because they lacked one or more of these substances, which generally are considered inimical to life and growth of the amoebae. The following experiments show that there is some truth in this supposition.

Experiment 9.—From a three-days-old culture the upper half of the fluid is carefully pipetted off (without disturbing the deposit) : it contains 1 amoeba per c.mm. (A) ; the lower half, well shaken, contains 600 amoebae per c.mm. (B). With this material, 7 tubes

are inoculated in the following way : tube 1 receives 20 drops of B, 40 dr. fresh serum-Ringer, none of A ; tubes 2, 3, 4 receive 1 drop of B, 40 dr. fresh serum-Ringer, 19 dr. of A ; tubes 5, 6, 7 receive 1 drop of B, 59 dr. fresh serum-Ringer, none of A. So tubes 2, 3, 4, 5, 6 and 7 started with the same number of amoebae, but 2, 3 and 4 with 20/60 old medium, whereas 5, 6 and 7 contained 1/60 old medium only. Tube 1 served as a control for the viability of the strain. All the tubes received starch and $CaCO_3$. The result was :

Tube	1	2	3	4	5	6	7	
at outset ...	200	10	10	10	10	10	10	amoebae per c.mm.
after 1 day...	73	3	12	10	0	0	0	" "
after 2 days	374	28	21	29	0	0	0	" "
" 3 "	258	9	7	20	0	0	0	" "
" 4 "	245	20	5	13	0	0	0	" "
" 7 "	—	—	—	—	0	0	0	" "

— = no count.

Although tube 1, as well as tubes 2, 3 and 4, yielded rather poor cultures, it cannot be denied that a liberal amount of old medium seems to favour life and growth of the amoebae. In another experiment, one-fifth only of old medium was added. It resulted as follows :--

Experiment 10.—A and B were prepared as in the previous experiment ; A contained 6 amoebae per c.mm., B 900 per c.mm. Again 7 tubes were inoculated in the following way : tube 1 received 12 drops of B, 48 dr. fresh serum-Ringer, none of A ; tubes 2, 3 and 4 received 1 drop of B, 48 dr. fresh serum-Ringer, 11 drops of A ; tubes 5, 6 and 7 received 1 drop of B, 59 dr. fresh serum-Ringer, none of A. All the tubes received starch and $CaCO_3$ as usual. The result was :

Tube	1	2	3	4	5	6	7	
at outset	180	15	15	15	15	15	15	amoebae per c.mm.
after 1 day	228	2	3	2	0	0	0	" "
" 2 days	568	3	11	17	0	0	0	" "
" 3 "	1,142	4	83	16	—	—	—	" "
" 4 "	—	0	587	29	0	0	0	" "
" 5 "	—	0	1,204	4	0	0	0	" "

— = no count.

Again, the tubes with much old medium did better than those with little, but within that group the individual tubes differ greatly, although I had endeavoured to start them under identical conditions. Similar major or minor differences may be seen in many experiments with this amoeba. That is the reason why I usually prepared three parallel tubes, or two at least, if enough material was not available.

Experiment 11.—In this experiment part of the deposit accidentally got mixed up with A, so that A contained 143 amoebae per c.mm. and B contained 1,285 amoebae per c.mm. Five tubes were inoculated thus : tube 1 received 20 drops of B, 40 drops fresh serum-Ringer, none of A ; tubes 2 and 3 received none of B, 40 drops fresh serum-Ringer, 20 drops of A ; tubes 4 and 5 received 2 drops of B, 52 drops fresh serum-Ringer, none of A. Hence, tubes 2, 3, 4 and 5 all began with 48 amoebae per c.mm., but 2 and 3 had 20/60 old medium, as against 4 and 5 with 2/52 old medium. Starch as usual ; no $CaCO_3$ was added, as the strain had proved not to need it. The result was :

Tube ...	1	2	3	4	5	
at outset... ...	428	48	48	48	48	amoebae per c.mm.
after 1 day ...	493	3	1	0	0	" "
" 2 days ...	578	8	2	0	0	" "
" 3 " ...	926	41	4	0	0	" "
" 4 " ...	833	73	3	0	0	" "
" 5 " ...	—	731	0	0	0	" "

— = no count.

Obviously a liberal amount of old medium may save the cultures with a small inoculum, which otherwise are doomed to death ; but it does not do so in every case. The question arises as to which constituent of the old medium does save the amoebae. Is it the bacteria, or the dissolved products of metabolism of the amoebae and the bacteria ? In any case, a small amount of one or the other is not sufficient ; cultures with a small inoculum get both, and yet they die. In order to solve this problem the following experiment was made.

Experiment 12.—A culture, five days old, with 1,670 amoebae per c.mm., furnished the inocula. To activate the slightly inoculated tubes, I used the fluid contained in three tubes that had served for another experiment with the same strain. These tubes had been slightly inoculated, and no amoebae had been found in them for 7 consecutive days. To separate bacteria and dissolved substances centrifuging was preferred to filtration, because filtration may remove not only the bacteria, but part of the dissolved matter. The fluid contained very numerous bacteria. It was centrifuged for one hour at 3,000 revolutions per minute. Then the upper half was carefully pipetted off (A) ; the deposit was dissolved again in the lower half by shaking (B). A contained a few bacteria, B very many ; both contained the same dissolved matter in equal quantities. Six tubes were inoculated thus :

	Inoculate with amoebae	B	A	Fresh serum-Ringer
Tube 1 ...	15 drops	—	—	45 drops
Tubes 2, 3, 4	1 drop	14 drops	—	45 ,,
,, 5, 6 ...	1 ,,	—	14 drops	45 ,,

Starch in all the tubes.

The result was :

Tube	1	2	3	4	5	6	
at outset ...	417	28	28	28	28	28	amoebae per c.mm.
after 1 day...	622	10	4	9	0	0	,, ,,
,, 2 days	1,487	92	97	374	0	0	,, ,,
,, 3 ,,	1,352	1,045	850	2,822	0	0	,, ,,
,, 4 ,,	800	1,632	2,023	1,020	—	—	,, ,,
,, 5 ,,	—	—	—	—	0	0	,, ,,
,, 7 ,,	121	229	349	105	0	0	,, ,,
,, 9 ,,	148	13	52	39	0	0	,, ,,
,, 11 ,,	1	0	0	0	0	0	,, ,,
,, 14 ,,	—	—	—	—	0	0	,, ,,

— = no count.

Evidently the ' activating factor ' present in the old medium is insoluble ; it is contained in the deposit, obtained by centrifuging. This deposit consisted mainly of bacteria ; a few rice-starch granules were also present. It is very improbable that this rice starch has played any rôle, as the quantity present in B is very small in relation to the quantity of fresh rice starch that was added later to all the tubes, including 5 and 6. Moreover, the old medium used in experiments 9 and 10 contained no rice starch, and yet proved effective. The only remaining conclusion is that the great quantity of bacteria added to tubes 2, 3 and 4 have saved the cultures. In the dosage of the bacteria I apparently have had a lucky hand—cultures with more than 2,000 amoebae per c.mm. are very rare.

In the next experiment I obtained the bacteria in a somewhat different manner.

Experiment 13.—From one culture-tube four new ones are inoculated : I, II and III with the upper part of the fluid, i.e., with bacteria only, not with amoebae ; tube IV was inoculated with the deposit. Three days afterwards, I, II and III showed no amoebae, bacterial growth in them was abundant ; tube IV then contained 1,496 amoebae per c.mm. The contents of I, II and III were mixed and centrifuged for one hour at 3,000 revolutions per minute ; then the upper half was pipetted off without disturbing the deposit (A), and the lower half rendered homogeneous by shaking (B). Ten tubes were inoculated thus :

	From IV	A	B	Fresh serum-Ringer
Tube 1	15 drops	—	—	45 drops
Tubes 2, 3, 4 ...	1 drop	14 drops	—	45 ,,
,, 5, 6, 7 ...	1 ,,	—	14 drops	45 ,,
,, 8, 9, 10 ...	1 ,,	—	—	59 ,,

Rice starch in all the tubes.

The result was :

Tube	1	2	3	4	5	6	7	8	9	10	
at outset	374	25	25	25	25	25	25	25	25	25	amoebae per c.mm.
after 1 day	103	0	0	0	0	0	2	0	0	0	,, ,,
,, 2 days	289	0	0	0	0	0	0	—	—	—	,, ,,
,, 3 ,,	513	0	0	0	0	16	17	0	0	0	,, ,,
,, 4 ,,	510	0	0	0	0	182	216	—	—	—	,, ,,
,, 5 ,,	299	0	0	0	0	510	677	0	0	0	,, ,,
,, 8 ,,	299	0	0	0	0	112	252	0	0	0	,, ,,

— = no count.

Here, again, two out of the three tubes with a small inoculum which received many bacteria did very well, all the others perished.

In spite of the contradictory results in some sets of parallel tubes, as observed in the experiments mentioned above (e.g., 2 against 3 and 4 in the last experiment), the conclusion is justified that a liberal amount of bacteria is a necessary condition for the life of *D. fragilis* under the circumstances of the experiments. Experiment 12 is of special importance in this respect. The bacteria were taken from three tubes in which the cultures had failed as far as amoebae were concerned ; during 7 days the bacteria multiplied, and then, because of their great numbers, they could serve as an activator for other cultures. The second conclusion is that even temporary scarcity of bacteria kills a culture of *D. fragilis*.

Can this biological character of the amoeba serve to facilitate the explanation of its spreading ? On the contrary. Assuming for a moment that in nature *D. fragilis* shows the same bacteria-' hunger ' as in the culture-tube, then we must conclude that outside the human bowel it can survive under special conditions only, viz., in places where an abundance of bacteria is to be found. This character can hardly favour its spreading nor explain its great prevalence.

It seems unlikely that *D. fragilis*, being so greatly dependent on bacteria, can ever become a primary tissue-parasite of man, nor that it will ever exchange the bacterial *dorado* of the human bowel for the barren, sterile human tissue. As far as I know, it has never been accused of any pathogenic action ; but this may also be due to the fact that its existence is hardly known to medical practitioners.

In order to test the hypothesis that there is a relationship between the presence of bacteria and the existence and absence of pathogenicity, it will first be necessary to ascertain how *Entamoeba histolytica* behaves in this respect.

SUMMARY

1. *Dientamoeba fragilis* is by no means a rare parasite in Holland. It was found in 36 per cent. of 80 asylum inmates, and was the commonest intestinal protozoon of this group.

2. Culture of intestinal protozoa is more successful if a small amount of $CaCO_3$ is added to the medium.

3. Cultures of *D. fragilis* were obtained from solid stools $6\frac{1}{2}$ hours after defaecation and from liquid stools $13\frac{1}{2}$ hours after defaecation.

4. The occurrence of living *D. fragilis* in solid stools is a common event.

5. Exposure to room-temperature (16–20° C.) adversely affects cultures of *D. fragilis*, the more so when the culture is young. In view of this fact, it seems improbable that *D. fragilis* is a free-living amoeba, only accidentally parasitizing man.

6. In cultures of *D. fragilis* there is a lag-phase, usually lasting more than one day.

7. Subcultures of *D. fragilis* need a large inoculum; if a small inoculum is applied the cultures invariably die within 24 hours. Such cultures may be saved if a liberal amount of (amoeba-free) old medium is added. The activating substance in the old medium can be separated from it by centrifugalization. The great amount of bacteria is the constituent of the old medium that saves cultures started with a small inoculum. *D. fragilis* can live in cultures only if there is a large number of bacteria present. Scarcity of bacteria kills a fresh culture within one day. It seems unlikely that this amoeba, being so greatly dependent upon bacteria, can ever become a primary tissue-parasite of man.

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STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

THE WARBLE FLIES OF THE GENUS *HYPODERMA*

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In an earlier paper (1935) I gave a short description (with illustrations) of the segmentation of the abdomen of the ♂ *Hypoderma bovis* de Geer, and of the ♂ and ♀ terminalia. In the present paper I am illustrating the diagnostic characters of the ♂ and ♀ terminalia of *H. lineatum*, *H. diana*, *H. tarandi*, *H. crossi* and *H. aeratum*, and giving short descriptions of the adults, together with some practical notes on their life-histories.

Hypoderma bovis de Geer. ADULTS. Dark, bee-like flies, about half an inch long. Front and cheeks covered with light yellow hairs. Presutural area of mesonotum with long yellow hairs, remainder with short black hairs, the shining black integument showing through and forming a broad dark band ; scutellum covered with yellow hairs. Basal third or so of abdomen with yellowish-white hairs; middle third with orange-tipped dark hairs, forming a conspicuous broad dark band ; apical third or more with bright orange hairs. All femora of legs black ; the tibiae dark brown, the distal half lighter brown ; tarsi light brown. The ♂ and ♀ terminalia are illustrated in my previous paper (1935).

NOTES. The eggs of *bovis* are laid singly out of sight near the base of a hair, especially on those of the hind leg about the knee, hock and stifle-joint ; they are also often laid on the flanks, region of rump, outer side of abdomen, outer thigh, on the forelegs about the knee and even on the dewlap. As the ♀ attaches only one egg at a time, returning again and again, and as it darts rapidly and violently at the hair, it strikes terror into cattle, often leading to a wild stampede which may result in injury. The first larva hatches in from 4 to 7 days, crawls down the hair and burrows into the skin, choosing a hair follicle for the point of entry. Penetration is completed in about six hours and produces considerable irritation, as evidenced by constant licking and rubbing ; small scabs form at the site. Very little is known of the migration of the larva from its point of entrance in the skin until its appearance in its final location in the back. One thing, however, is certain ; it does not go to the submucous coat of the oesophagus, as does the larva of *lineatum* ; some larvae have been recorded from the spinal canal. As *bovis* is the late warble fly, the adults are on the wing from late June to August, and may even be seen as late as September. The ♀ lives for about a week in warm weather, and for about three in cooler weather. The first stage larvae begin to appear in the skin of the back usually towards the end of February, and continue to appear throughout March to May. It should be

remembered that larvae may be found in the skin as late as August, as noted by MacDougall (1930), who found a larva of *bovis* nearing the third stage on August 9th in Scotland. The third stage larva remains in the skin of the back about 70–80 days ; it then drops out, usually during the daytime, crawls away and buries itself in the ground, where it slowly pupates. The pupal stage lasts about 5–8 weeks.

Hypoderma lineatum Villers. The heel fly. ADULTS. Also bee-like hairy species, but smaller than *bovis*. Front and cheeks covered with yellowish-white hairs. The mesonotum appears uniformally dark, but, when viewed with the head facing the observer, it will be noted that the anterior part is covered with light yellow hairs. Owing to the sparse covering of hairs, four shining lines are clearly visible. The base of the abdomen is covered with greyish-yellow hairs, the middle part is dark brown to black, and the end is covered with

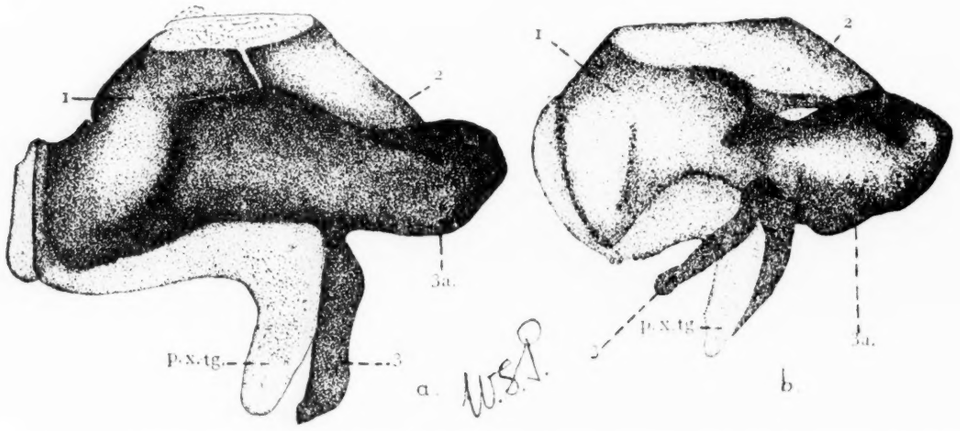


FIG. 1. *a*.—Tenth tergum of *tarandi*, showing ninth coxite and anal cercus in side view ; 1.—Tenth tergum ; 2.—Anal cercus ; 3.—Proximal segment of ninth coxite ; 3*a*.—Distal segment of same ; *p.x.tg*.—Anterior process of tenth tergum which articulates with side of ninth tergosternum ; *b*.—Tenth tergum of *lineatum*, showing ninth coxite and anal cercus in side view ; lettering as above.

bright orange hairs. The femora are dark brown to black, and the tibiae and tarsi are lighter brown. TERMINALIA. ♂. The ♂ terminalia are illustrated in figs. 1 ; 2. It will be noted that they are very similar to those of *bovis*. The anal cercus and distal segment of the ninth coxite (fig. 1, *b*) are longer than those of *bovis*, and the latter is more pointed. The struts of the phallosome are rather wider when seen in side view ; the paramere is very similar to that of *bovis*, but sternum 5 (fig. 2, *c*) is somewhat differently shaped, the lobes being more rounded. The ♀ terminalia are illustrated in fig. 3, and, though most of the sclerites are very similar to those of *bovis*, the sixth and seventh terga are a little different.

NOTES. *H. lineatum*, as contrasted with *bovis*, lays a number of eggs on a hair, and as many as 20 have been recorded. If the animal is standing, the fly usually settles momentarily and fixes its eggs towards the middle or end of the

hair, and as a rule does not disturb the animal. If the host is lying down, the fly settles on the ground in the shade of the hind leg, extends the long ovipositor, and lays the eggs on the hairs around the coronet, and also on other parts of the leg in proximity to the ground. Eggs are usually laid on hairs from the point of the ischium to the hock, the upper part of the udder, the point of the ischium to the patella, the flanks and sternum behind the elbow ; the most favourite site seems to be the hair on the back of the thigh just below the root of the tail. As the eggs are laid towards the end of the hair, they are as a rule much more visible than are those of *bovis*. The incubation period is 4–7 days. The first larva hatches

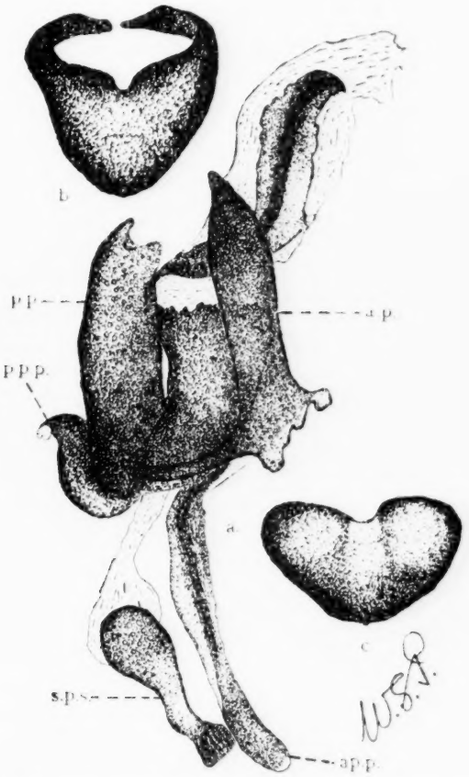


FIG. 2. *a*.—Phallosome and one paramere of *lineatum* in side view ; *a.p.*—Anterior part of paramere ; *ap.p.*—Apodeme of phallosome ; *p.p.p.*—Posterior process of phallosome ; *s.p.s.*—Sperm pump sclerite ; *b*.—Ninth tergo-sternum ; *c*.—Fifth sternum.

of its own accord, crawls down the hair, and enters the skin through a hair follicle ; the time for penetration is probably the same as that in the case of the larva of *bovis*. Those larvae nearest the skin hatch first, and most of those from one hair enter the skin at the same spot. Hadwen (1923) states that in adult cattle there is a reaction in the skin which leads to the death of many larvae, and that this probably explains why adult cattle always have fewer warbles than young animals. A swelling develops at the site of the entry of the larvae, and, as much serum exudes from the punctures, scabs form, and when rubbed by the animal the skin condition suggests mange or cowpox, as pointed out by Hadwen. A pustular condition may develop, following secondary

infection. The so-called ' hypoderma rash ' is then, for the reasons noted, always more marked in the case of *lineatum* than *bovis*. The exact route of the first stage larva (and the time it takes) from the point of entrance in the skin to the submucous coat of the oesophagus is not definitely known. Laake (1921), who considers that *lineatum* has five larval stages, believes that the first larva moults on its way to the oesophagus, where it is found in the submucous coat as the second stage. After remaining in the submucous coat of the oesophagus

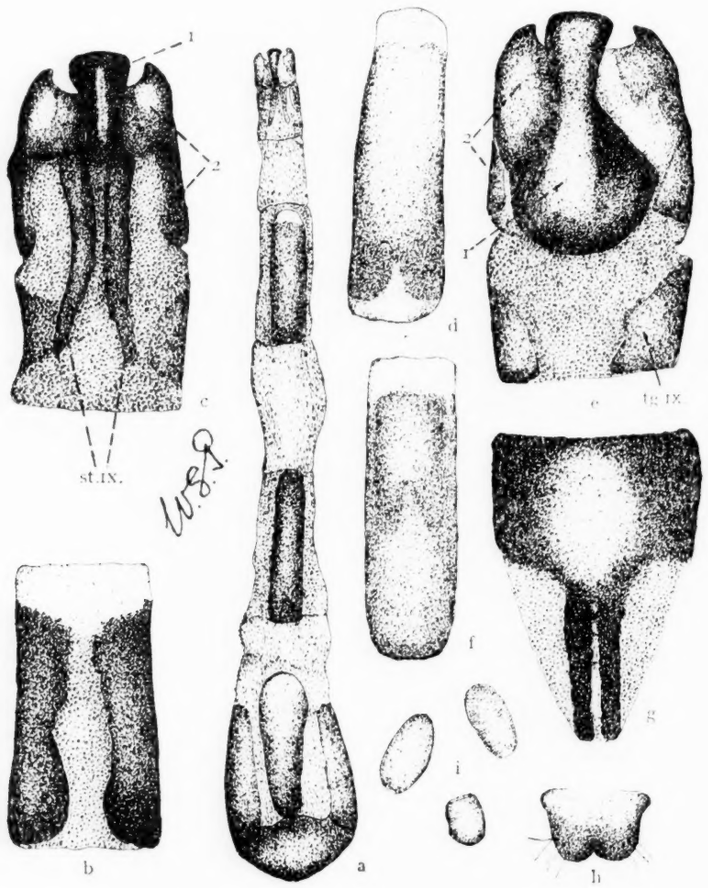


FIG. 3. *a*.—Ventral view of fully extended ovipositor of *lineatum* ; *b*.—Seventh tergum ; *c*.—Ventral view of end of ovipositor ; 1.—Tenth tergum ; 2.—Anal cercus ; *st.ix*.—Ninth sternum ; *d*.—Seventh sternum ; *e*.—Dorsal view of end of ovipositor ; *tg.ix*.—Tenth tergum ; *f*.—Sixth sternum ; *g*.—Sixth tergum ; *h*.—Palps of ♀ ; *i*.—Spermathecae.

for about 2–3 months, the first larva starts on its second migration to the back. Laake claims that his second stage larva moults on the way and the so-called spineless third stage larva is formed. On reaching the skin of the back, the larva is actually still in its first stage, and it now undergoes two moults in the warble, finally reaching the third stage. According to Laake, the stages in the warble are the fourth and fifth.

Hadwen has traced the second migration of the first stage larva of *lineatum* in Canada, and the following is a summary of his observations : (*a*) The larvae

in the oesophagus gradually work their way to the diaphragmatic end of the organ. (*b*) They may follow the posterior borders of the ribs under the pleura. (*c*) They may enter the spinal canal. (*d*) There is some evidence that the posterior foramen is the means of egress and exit. (*e*) Finally the larvae follow connective tissue closely.

The present writer has all along maintained with Gedoelst (1922) and others that there are only three larval stages in *Hypoderma*. The following notes in support of this view were written ten years ago. The flaw in Laake's contention that there are five larval stages in *Hypoderma* lies in the fact that there is no proof that the first stage larva is distinct from his so-called second stage found in the submucous coat of the oesophagus ; no moult has been observed from the first to the second stage. The argument in support of the view that there are two distinct stages before the so-called spineless third stage is not convincing, owing to the fact that there is a great variation in the arrangement of the spines in all stages of the larva of *Hypoderma* if a long series is examined critically, and this is specially so in the case of the third stage (spineless) larva. Dr. Stewart MacDougall has kindly given me permission to note the result of the examination by Mr. J.W. M'Hardy of a large number of larvae of *lineatum* from oesophagi from carcases at the abattoirs in Edinburgh, which, as far as I know, has not been recorded. The larvae were all the so-called spineless stage, and were collected from January 9th to March 2nd, 1925. Mr. M'Hardy noted that the arrangement and number of spines showed marked variation on the body segments. On some larvae very few spines were found, while on others they were present in rows, similar to those depicted by Laake in his second stage. All gradations were observed between the extremes, and where spines were not numerous they always occupied positions which suggested that they were the remains of the regular arrangement, as in larvae with a complete set of spines. This type of larva was found right up to the time when they usually migrate to the skin ; three such larvae were found in the skin of the back. Mr. M'Hardy came to the conclusion that all the larvae he examined represented the so-called third stage of Laake ; they showed variations from those described by Laake as follows: (*a*) In the size of the cephalopharyngeal skeleton and mouth-parts; (*b*) In the presence of spines on the body segments. The so-called spineless third stage larva is, therefore, in no sense spineless if a long series is examined critically. There is no proof that it is anything more than the first stage larva. The results of these observations of Mr. M'Hardy do not substantiate the view that there are five larval stages in *Hypoderma*.

Recently, Knipling (1935) has re-studied the whole question of the number of larval stages in *H. lineatum*, and as a result of his painstaking observations on more than 1,000 larvae from the oesophagi of cattle he has confirmed the opinions of those who have always maintained that there are only three larval stages. For details of his arguments and explanation of the changes that take place as the first stage larva gradually increases in size, the reader should consult

his paper. The three larval stages of *bovis* and *lineatum* may be summarized as follows :—

First Stage Larva. Hatches of its own accord, neither friction nor moisture being necessary. Crawls down a hair and penetrates skin at its base. In the case of *lineatum* it travels by an unknown route to the oesophagus, where it remains for from 2 to 3 months ; during this time it increases in length. It now migrates to the skin of the back, where it moults to the second stage. The corresponding larva of *bovis* does not go to the oesophagus, but it may be found in the spinal canal. The method by which the larva punctures the skin, and the time it takes to do so, are not known.

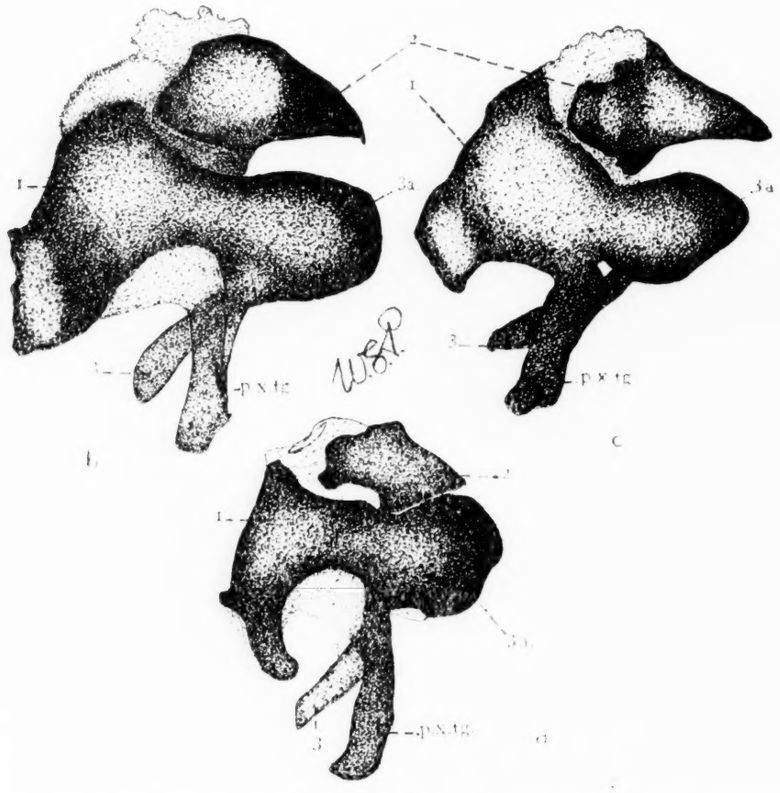


Fig. 4. *a*.—Tenth tergum of *crossi*, showing ninth coxite and anal cercus in side view ; lettering as in fig. 1, *a* ; *b*.—Tenth tergum of *diana*, also showing ninth coxite and anal cercus in side view ; *c*.—Tenth tergum, ninth coxite and anal cercus of *aeratum* in side view.

Second Stage Larva. The second stage larva becomes encysted in the skin of the back, where it moults again to the third stage.

Third Stage Larva. Remains in the warble until mature, when it crawls out and pupates in the ground.

In the British Isles the larvae of *lineatum* begin to appear in the submucous coat of the oesophagus in August, they increase to a maximum in November, and gradually decrease in numbers in December and January. In these months they first begin to appear in the skin of the back ; in February they become

common in the skin, and the number in the oesophagus now shows a marked decrease. In March hardly any larvae are left in the oesophagus, and from April to July no more larvae can be found in the oesophagus. The third stage larvae of *lineatum* begin to leave the skin somewhere between March 1st and March 15th and continue to do so until about May. It will be noted, then, that early in the year (end of January) the larvae of *lineatum* begin to appear in the skin, the warble then being about the size of a pea. The first stage larva

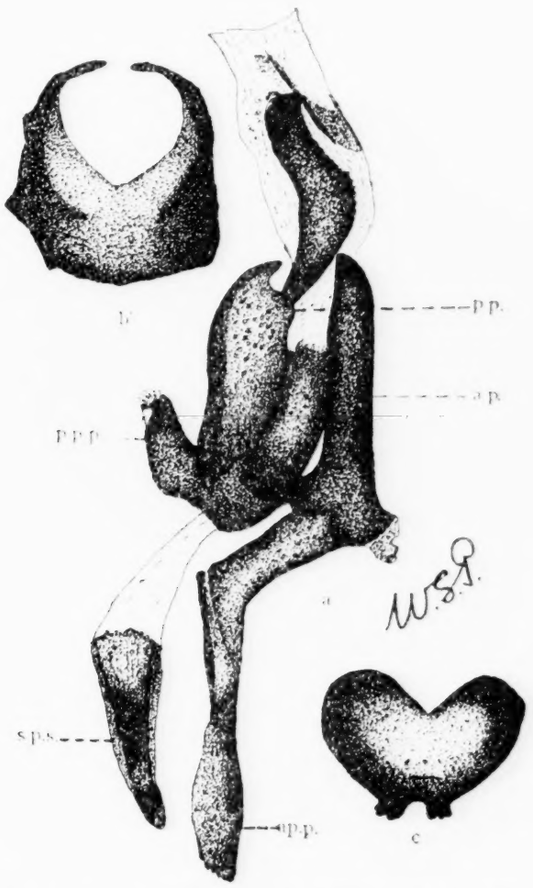


FIG. 5. *a*.—Phallosome and one paramere of *diana* in side view; lettering as in fig. 2, *a*; *b*.—Ninth tergo-sternum; *c*.—Fifth sternum.

completes its moults in the skin, and reaches full growth in about 40 days; Bishopp and his co-workers give the minimum period in America as 35 days, while Obitz (1935) gives an average of 56 days in Poland. The pupal stage lasts from 3 to 5 weeks. The adults are on the wing in May and June; a few may even be seen in the early autumn.

Hypoderma diana Brauer. ADULTS. Smaller, bee-like species, resembling *lineatum*. Front and cheeks covered with silky hairs. The mesonotum is dark, with greyish-yellow hairs and with four narrow black polished lines, as in *lineatum*. The abdomen is dark, the hairs at the base being brassy-yellow, while

at the extremity they have a redder tinge. The scutellum has two large rounded lobes on each side of the middle line, and a much less marked one at each side. TERMINALIA. ♂. The ♂ terminalia are illustrated in figs. 4; 5. The anal cercus of *diana* (fig. 4, *b*) is relatively small and pointed when seen in side view, and the distal segment of the ninth coxite is broadly rounded at the end. The phallosome (fig. 5, *a*) is structurally similar to that of *bovis* and *lineatum*, and the struts are fairly wide; there is a well-marked area of chitinization between the end of the struts in the middle line, as shown in fig. 5, *a*. The two parts of the

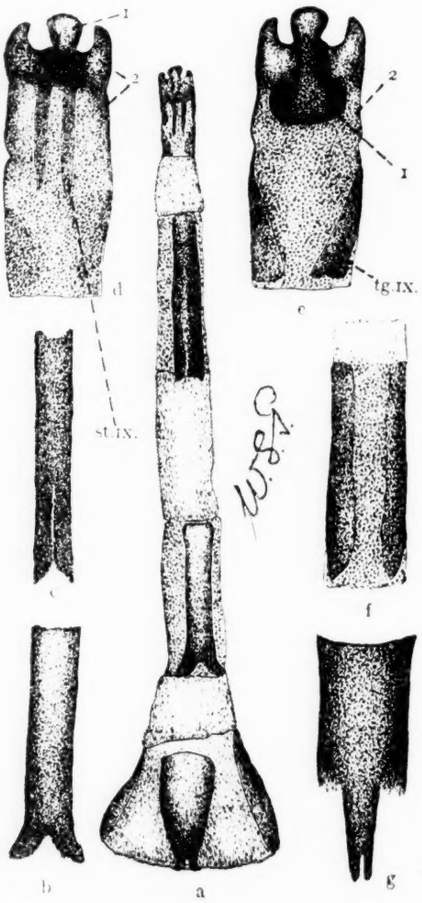


FIG. 6. *a*.—Ventral view of fully extended ovipositor of *diana*; *b*.—Sixth sternum; *c*.—Seventh sternum; *d*.—Ventral view of end of ovipositor; lettering as in fig. 3, *c*; *e*.—Dorsal view of end of ovipositor; lettering as in fig. 3, *e*; *f*.—Seventh tergum; *g*.—Sixth tergum.

paramere are very similar to those of *bovis* and *lineatum*. The fifth sternum (fig. 5, *c*) is rather like that of *lineatum*. ♀. Fig. 6. The ♀ terminalia are illustrated in fig. 6; here again it should be noted that they are very similar to those of *bovis* and *lineatum*.

NOTES. *H. diana* is the warble fly of the red deer of Europe. At present very little is known of its life-history. The adults are on the wing in May and June. It is assumed that the eggs are laid on the hair of the host, and that the

first stage larvae burrow into the skin, as do those of *bovis* and *lineatum*, and that, like the former, the larva does not go to the oesophagus. The first stage larvae are found in warbles in the skin from Jaunary onwards. Parnell and his co-workers (1932) record having noted 37 larvae in a piece of skin measuring 12 inches by 6 inches.

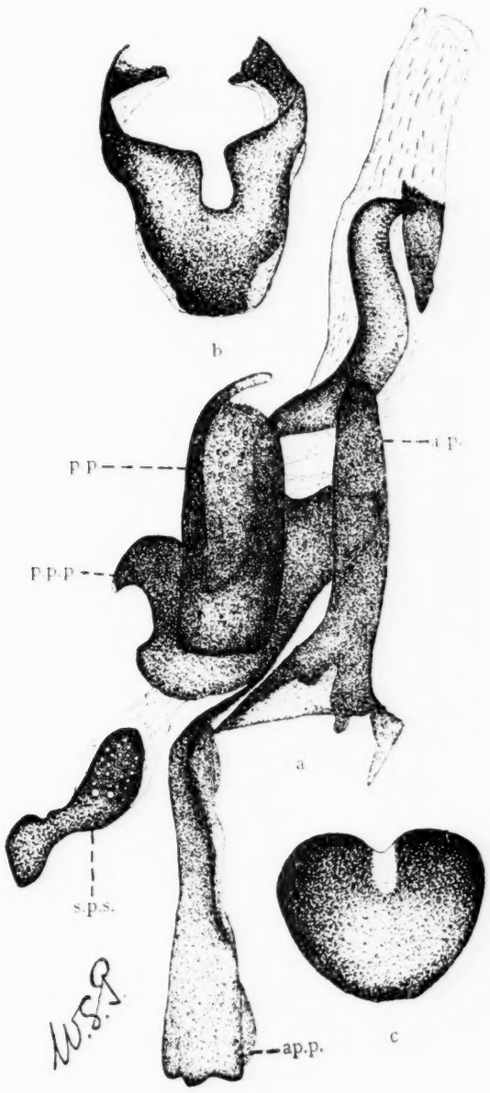


FIG. 7. *a*.—Phallosome and one paramere of *tarandi* in side view ; lettering as in fig. 2, *a* ; *b*.—Ninth tergo-sternum ; *c*.—Fifth sternum.

Hypoderma (Oedemagena) tarandi L. ADULTS. A large bee-like species with the characteristic *Hypoderma* facies. The vertex is black, and the front and cheeks are covered with silky hairs. The palps are visible and are separated. The mesonotum is covered with yellow hairs, the middle sparsely with short dark hair, and appears to form a dark band with four shining lines. The abdomen is mainly covered with long red hair, the base with yellow hairs ; the ovipositor

is very long. The legs are very hairy, the femora and most of the tibiae are black, except the hind pair, which are mainly light brown, especially posteriorly ; the tarsi are lighter brown. TERMINALIA. ♂. The ♂ terminalia are illustrated in figs. 1 ; 7. The anal cercus (fig. 1, *a*) is long and rather bluntly pointed ; the distal segment of the ninth coxite (fig. 1, *a*) is very long and bluntly pointed.

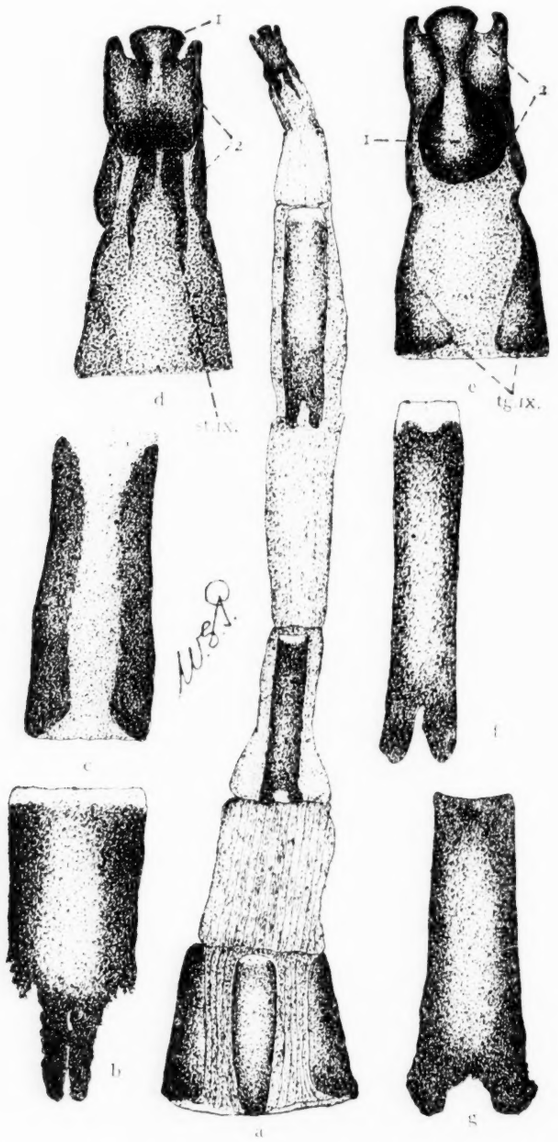


FIG. 8. *a.*—Ventral view of fully extended ovipositor of *tarandi* ; *b.*—Sixth tergum ; *c.*—Seventh tergum ; *d.*—Ventral view of end of ovipositor ; lettering as in fig. 3, *c* ; *e.*—Dorsal view of end of ovipositor ; lettering as in fig. 3, *e* ; *f.*—Seventh sternum ; *g.*—Sixth sternum.

The phallosome is structurally similar to that of the other species, but is longer, especially the distal membranous part. There is a well-marked chitinous area between the struts at the end, as in the case of the phallosome of *diana*. ♀. The ♀ terminalia are illustrated in fig. 8. A comparison of the various sclerites

with those of the other species leaves little doubt that *tarandi* is closely related to them.

NOTES. The following notes on the life-processes of *tarandi*, as far as is known at present, are taken from Bergmann (1917), Hadwen and Palmer (1922), Hadwen (1926) and Natvig. *H. tarandi* lays its eggs from June to September on the hairs of the legs, flanks and brisket of reindeer when lying down. When the reindeer are standing, the eggs are mainly laid about the stifle-joint and elbow. It is interesting to note that the great length of the ovipositor is correlated with the attachment of the eggs to the hairs of the woolly

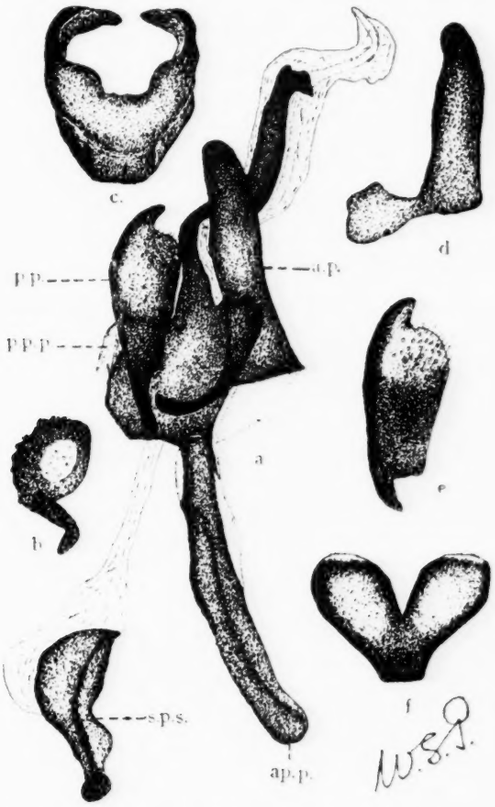


FIG. 9. *a*.—Phallosome and one paramere of *crossi* in side view; lettering as in fig. 2, *a*; *b*.—Another view of sperm pump sclerite; *c*.—Ninth tergo-sternum; *d*.—Anterior part of right paramere; *e*.—Posterior part of right paramere; *f*.—Fifth sternum.

down of the undercoat, the hairs of the outer being very long. Hadwen states that a ♀ can lay more than 500 eggs, and he has counted 796 eggs on one half of a reindeer's body. The egg hatches in from 6 to 7 days. The first larva, on hatching, penetrates the skin and makes its way in the muscular sheaths to the back, the exact route not being known. Hadwen has shown that the larva does not go to the oesophagus. The first stage larvae begin to reach the skin of the back by about the end of September, and by October the holes in the skin increase in size. The larvae remain in the skin until May and most of June. It is not uncommon to find as many as 1,000 (or even more) larvae in the skin of the back of one animal.

Hypoderma crossi Patton. ADULTS. Smaller, lighter-coloured species, not bee-like, and sparsely covered with yellow hairs. The front and cheeks are clothed with short silky hairs. The mesonotum is dark grey and is covered with short yellow hairs; there are four rather inconspicuous dark stripes, the outer pair longer and better marked, but not shining. The scutellum ends in a pair of rounded, shining projections or bosses, one on each side of

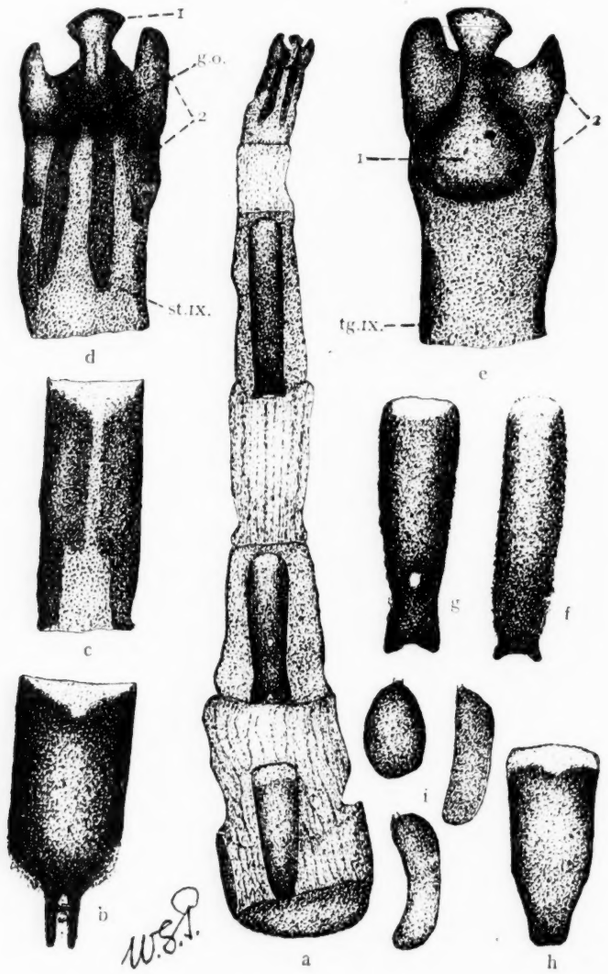


FIG. 10. *a*.—Ventral view of fully extended ovipositor of *crossi*; *b*.—Sixth tergum; *c*.—Seventh tergum; *d*.—Ventral view of end of ovipositor; lettering as in fig. 3, *c*; *e*.—Dorsal view of end of ovipositor; lettering as in fig. 3, *e*; *f*.—Seventh sternum; *g*.—Sixth sternum; *h*.—Fifth sternum; *i*.—Spermathecae.

the middle line, and also a conspicuous one at each side. The abdomen is lighter and is covered with short golden hairs; a large light-bluish-grey patch on each side of middle line on terga 3 and 4, and also on each side of tergum 5, better marked in the ♀, giving the abdomen a tessellated appearance. TERMINALIA. ♂. The ♂ terminalia are illustrated in figs. 4; 9. The anal cercus (fig. 4, *a*) is very short and pointed; the distal segment of the ninth coxite (fig. 4, *a*) is

also short and broadly rounded at the end. The phallosome (fig. 9, *a*) is shorter, and the struts are narrower, ending in a marked hooked point; otherwise its structure is exactly similar to that of the other species. The two parts of the paramere are also very similar to those of the other species. ♀. The ♀ terminalia are illustrated in fig. 10; it will be noted that the various sclerites are very like those of the other species.

NOTES. The egg-laying habits of this species are not yet known. At present it is only known from north-west India, where its larva is a common parasite of the goat. Mr. Cross, who originally sent me the adults, which he hatched out from larvae dropping out of warbles in the skins of goats, also sent me some larvae which had come from the skins of cattle. The common warble

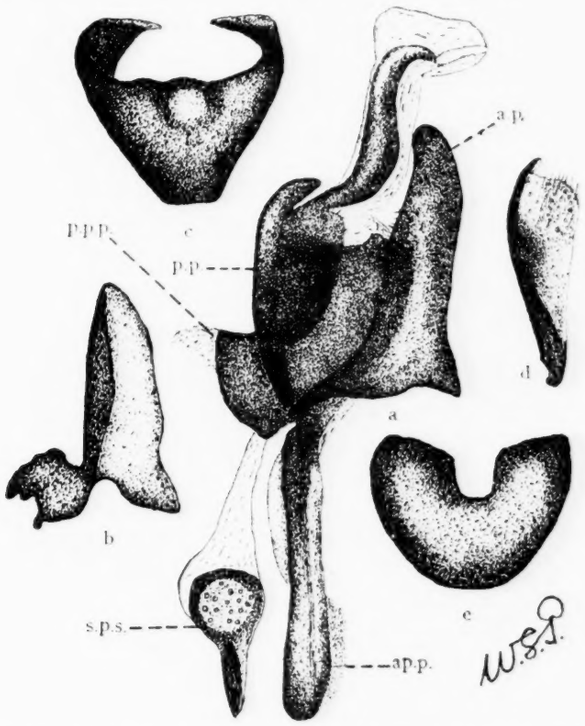


FIG. 11. *a*.—Phallosome and one paramere of *aeratum* in side view; *b*.—Anterior part of right paramere; *c*.—Ninth tergo-sternum; *d*.—Posterior part of right paramere; *e*.—Fifth sternum.

fly of cattle in north India is, however, *lineatum*. Bhatia (1934) suggests that the eggs are laid on the long hairs on the sides of the body. As the ovipositor is not very long, as is that of *tarandi*, I think that it is much more likely that the eggs are laid on the hairs of the legs, when the animals are either lying down or standing up. The adults are on the wing from about the middle of February to April. The first stage larvae usually begin to appear in the skin of the back in July and continue to do so through August and September. They are mature by December, towards the end of which, and in January and February, they leave the host to pupate in the ground. It is not known whether the larvae migrate to the oesophagus, but they probably do not.

Hypoderma aeratum Austen. ADULTS. A small species, similar to *crossi* and not bee-like. Front and cheeks clothed with white hairs. The mesonotum is dark and olive-coloured, and is covered with fine short brownish hairs in the ♂ and yellowish hairs in the ♀; there are four faint dark longitudinal stripes, as in *crossi*. The scutellum also has similar round black bosses, with a tuft

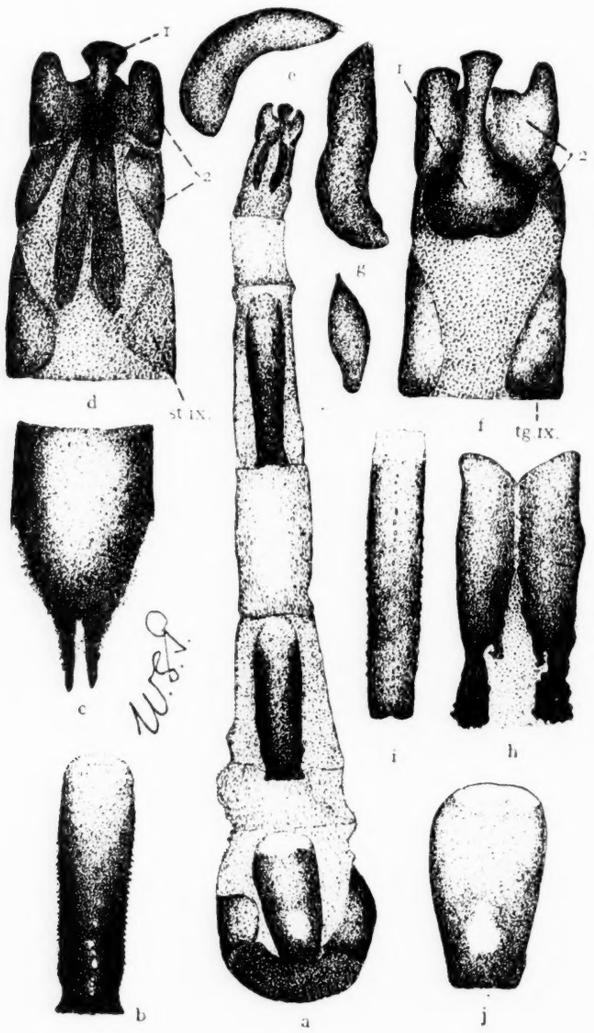


FIG. 12. *a*.—Ventral view of fully extended ovipositor of *aeratum*; *b*.—Sixth sternum; *c*.—Sixth tergum; *d*.—Ventral view of end of ovipositor; lettering as in fig. 3, *c*; *e*.—One spermatheca; *f*.—Dorsal view of end of ovipositor; lettering as in fig. 3, *e*; *g*.—The two other spermathecae; *h*.—Seventh tergum; *i*.—Seventh sternum; *j*.—Fifth sternum.

of yellow hairs between them. The abdomen is covered with yellow hairs and is without the tessellated appearance of that of *crossi*.

TERMINALIA. ♂. The male terminalia are illustrated in figs. 4; 11. On comparing the anal cercus and distal segment of the ninth coxite with those of *crossi*, it will be noted that the anal cercus of *aeratum* is longer and wider, and that the distal segment of the ninth coxite is narrower and more pointed

than that of *crossi*. The phallosome (fig. 11, *a*) is similar structurally to that of the other species, and especially to that of *crossi*; it differs, however, in that the struts do not end in hook-like points. The anterior paramere (fig. 11, *a*, *c*) is distinctly wider and generally larger than that of *crossi*, and sternum 5 (fig. 11, *e*) is, on the whole, broader, with less defined lobes. ♀. The ♀ terminalia (fig. 12) are very similar to those of the other species.

NOTES. *H. aeratum* is at present only known from the Island of Cyprus, where its larva is a parasite of goats. Practically nothing is known of its life-history.

There are many other species of *Hypoderma* which have been recorded, some only in the larval stage and some only as adults. Although I have studied the adults of *H. actaeon* Brauer, whose larva is parasitic in the skin of the red deer in Europe, I have not had an opportunity of examining the terminalia either of this species or of any of the others which have been described. I wish to thank Dr. Szilady for a gift of the ♂ and ♀ of *H. diana*, which enabled me to study the terminalia of this species. The specimens of *H. aeratum* Austen were given me by the late Mr. Noel Pillers; they were bred out from larvae by Mr. H. M. Morris, M.Sc., Government Entomologist, Cyprus. For specimens of *H. tarandi* I am indebted to Mr. Emory C. Cushing.

A comparative study of the illustrations of the ♂ and ♀ terminalia of the six species dealt with in this paper leaves no doubt that the genus is a homogenous one, the species being very closely related to each other. I can, therefore, see no reason whatever for splitting the genus into smaller genera, especially when the characters used to define these genera are based on incomplete study of the species. It will be remembered that Latreille erected the genus *Oedemagena* for the warble fly of the reindeer. The genus is characterized by the presence of small (separated) palps, whereas these structures are said to be absent in the restricted genus *Hypoderma*. This, however, is not the case, for all the species of *Hypoderma* also possess palps which are for the most part smaller and fused (fig. 3, *h*); they are as a rule not visible, but can be dissected out of the head in a caustic potash preparation. *Tarandi* on the characters of the terminalia is a typical *Hypoderma*, and it is interesting to note that within the genus it is more closely related to *diana* than to the other species studied. Another character which has been used to split the genus is the presence or absence of bosses on the scutellum. Here again it is clear that this character has been incompletely studied. A comparative study of the scutellum in *Hypoderma* shows that, starting with *tarandi*, in which the bosses are absent, there are all gradations to the well-developed ones on the scutellum of *crossi* and *aeratum*; this character, then, is of no value for defining genera. As pointed out in my previous paper (1935), the genera *Cuterebra* and *Dermatobia* are related to *Hypoderma*, and all three are placed in the subfamily Hypodermatinae, in the family Muscidae. In my next paper in this series I shall deal with the bot flies of the subfamily Oestrinae.

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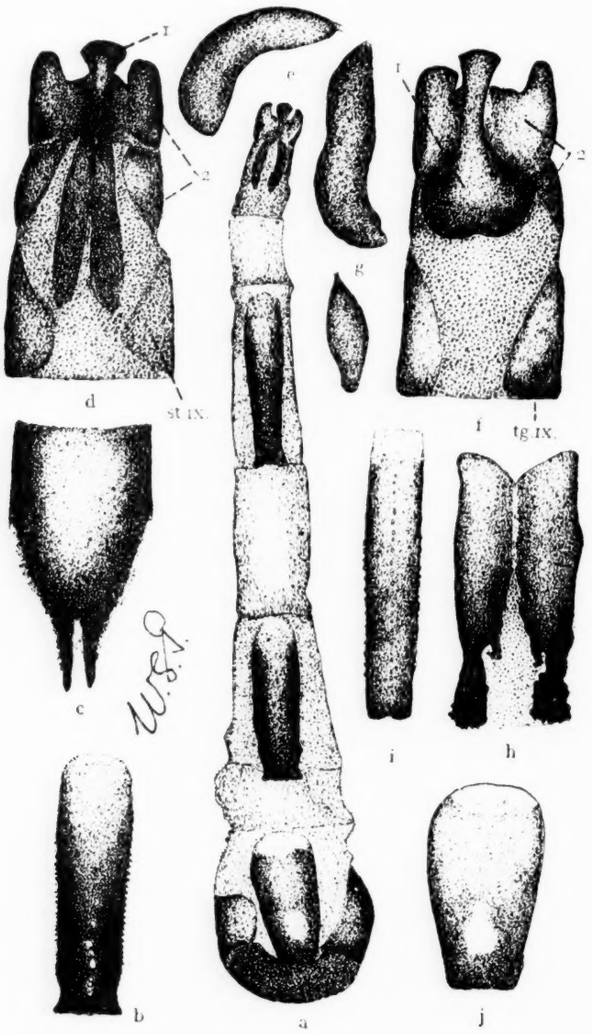


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(*To be continued*)

STUDIES ON THE HIGHER DIPTERA OF MEDICAL AND VETERINARY IMPORTANCE

A REVISION OF THE SPECIES OF THE GENUS *MUSCA*, BASED ON A COMPARATIVE STUDY OF THE MALE TERMINALIA

III.—A PRACTICAL GUIDE TO THE ETHIOPIAN SPECIES

(*Continued from Vol.* 27, *page* 430)

BY

W. S. PATTON

(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication* 17 *October*, 1936)

It is now ten years since I described the Ethiopian species of the genus *Musca* (1926); during this interval I have received fresh material, and I am now in a position to revise the species. I am indebted to Dr. A. R. D. Adams for a large collection from Mauritius, which has enabled me to settle the identity of *albomaculata* Macq. In addition, I have had large collections from Mr. A. Cuthbertson (including many larvae), from Southern Rhodesia; from Mr. Symes, from Kenya; from Mr. Potts, from Tanganyika; from Dr. Lamborn, from Nyasaland; from Mr. Gibbins (including larvae), from Uganda; and from Mr. Munro, from South Africa. I wish to take this opportunity of thanking these gentlemen for the great trouble they have taken in getting the collections together. In order to save space, I do not propose to re-describe those species which are also found in the Palaearctic region. Notes on, and illustrations of, their ♂ terminalia, as well as illustrations of the posterior spiracles of the third stage larva, will be found in the paper (1933*b*) dealing with the Palaearctic species, which will be referred to here as Part 2 (Pt. 2 for short). Illustrations of the ♂ terminalia of some of the others will be found in the paper published in 1932, which will be referred to as Part 1 (Pt. 1 for short). The species of the Ethiopian region, like those of the other regions, fall into the three groups *domestica*, *sorbens* and *lusoria*. As in Pt. 2, I shall give only brief descriptions of the species, noting in particular those characters which are of use in identification; keys to both sexes will be found at the end of this paper.

DOMESTICA GROUP

Musca domestica L. I have repeatedly been asked whether *domestica* L. occurs in Africa. My answer to this question is that I have no proof either that it has established itself at ports visited by ships from Europe, or that it has spread inland and become established there. I have no doubt that it is regularly carried on ships to both eastern and western ports of Africa. I have examined

large collections of house- and bazaar-frequenting species of *Musca* from Southern Rhodesia, Nyasaland, Tanganyika, Kenya and Uganda, and, except for odd, doubtful specimens, I have not seen any series of the typical *domestica* L. which one would expect if it is established in these parts. In this connection, it is necessary to be quite clear as to what I mean by the ♂ *domestica* L. In the typical European *domestica* the vertex (the entire space between the eyes) is very wide, almost equal to the width of an eye (fig. 1, A), whereas in the common tropical house fly, *M. vicina*, the vertex is about half that of *domestica* (fig. 1, B). Although the vertex of *vicina* Macq. varies in width, it is never equal to that of the ♂ *domestica*. *Musca domestica* is undoubtedly established in South Africa, and appears to have spread inland, but how far I do not know. For the synonomy, descriptions of adults and illustration of the posterior spiracles of the third stage larva, see Pt. 2; for illustrations of the ♂ terminalia, see Pt. 1.

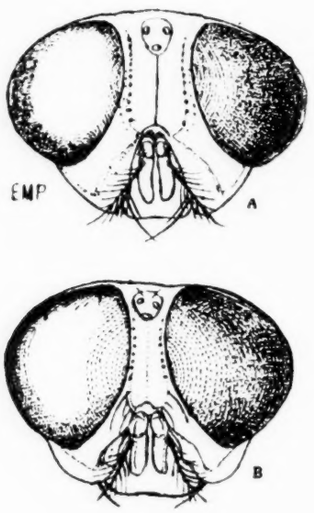


FIG. 1. A.—Front view of head of ♂ *Musca domestica*, to show width of vertex; B.—Front view of head of ♂ *M. vicina*; the vertex is about half the width of that of *domestica*.

Musca vicina Macquart. This is the common house and bazaar fly of tropical Africa; the vertex of the ♂ is illustrated in fig. 1, B. As well as breeding in decomposing animal matter and in horse and human faeces, it commonly breeds in cow-dung. For the synonomy, descriptions of adults, illustrations of the ♂ terminalia and posterior spiracles of the third stage larva, see Pt. 2.

Musca cuthbertsoni sp.nov. MALE. HEAD. Eyes bare, with slightly larger facets on the inner and upper surface. Vertex narrow, about one-seventh width of an eye; cheeks and lower vertex satiny. MESONOTUM. Four broad black stripes, the outer pair narrowly separated by a grey stripe, which is sometimes almost wanting posteriorly, so that the black stripes appear to be fused. ABDOMEN. Light orange; terga 1 and 2 orange, anterior

half or less black, with a median narrow black stripe; tergum 3 orange, with a broad median black stripe, extreme lateral edges of tergum with some silvery dusting; tergum 4 orange, also with a dark median stripe, a silvery stripe varying in width (sometimes wanting) on each side of median stripe, and silvery patch at edges of tergum; tergum 5 with a triangular median silvery patch, which appears dark in some lights, a broad silvery stripe on each side, and edges of tergum silvery.

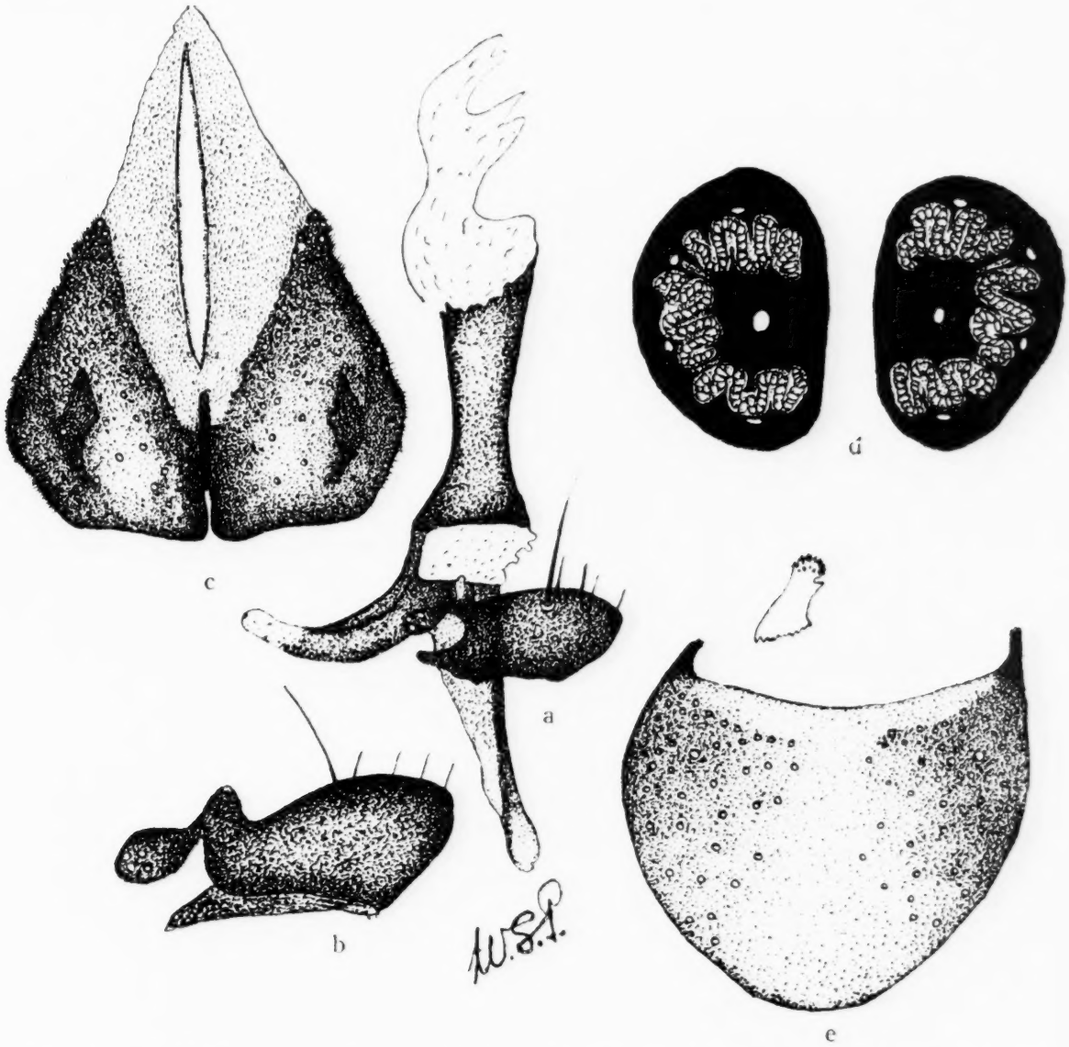


FIG. 2. *a*.—Phallosome and one paramere of *cuthbertsoni* in side view; *b*.—Left paramere; note the small, almost flat posterior part; *c*.—Anal cerci; *d*.—Posterior spiracles of third larva; *e*.—Fifth sternum, with one posterior process enlarged.

MALE TERMINALIA. Fig. 2. ANAL CERCI. Fig. 2, *c*. Very similar to those of other species of this group, the outer ends rounded and the inner not forming nipples. PHALLOSOME. Fig. 2, *a*. The chitinous portion is long and narrow, the posterior process short, rather narrow and not much expanded at the end. PARAMERE. Fig. 2, *a*, *b*. Anterior part wide, with 3–5 short

bristles and one longer one ; the posterior part is not raised and has the usual four sensory spines. FIFTH STERNUM. Fig. 2, *e*. The fifth sternum is wide, the postero-lateral processes rather narrow.

FEMALE. HEAD. Vertex wide, a little less than width of an eye ; vertical stripe dark brown and about three-quarters width of vertex ; outer vertical hairs in two rows ; cheeks satiny. MESONOTUM. Grey, with the usual four broad black stripes. ABDOMEN. Rather bright orange ; terga 1 and 2 orange, except middle of anterior border, which is black ; tergum 3 orange, with a broad black median stripe, which expands along posterior border forming a narrow dark band, yellowish admedian patches and margins yellow ; tergum 4 orange, with a slightly narrower median dark stripe, and a narrow dark posterior band, yellowish patches (or stripes) on each side of median stripe, and also at margins ; tergum 5 orange, with a narrow median dark stripe and yellow patches at sides.

EARLY STAGES. Oviparous, eggs laid in batches in organic matter and excrement of all kinds. THIRD LARVA. Anterior spiracles with 5–7 (usually 6) finger-like openings. POSTERIOR SPIRACULAR PLATES. Fig. 2, *d*. Rather wide and long, densely chitinized and close together; breathing slits long and wide.

NOTES. I have much pleasure in naming this species after Mr. Cuthbertson, whose work on the Diptera of Southern Rhodesia, and especially the higher forms, is well known. It is several years now since Mr. Cuthbertson sent me the adults as well as larvae, and, as it is related to *yerburyi* Patton and to *nebulo* F., both Mr. Cuthbertson and myself have referred to it under these names. It has only been possible by a comparative study of the ♂ terminalia of the three species to reach the final conclusion that this Rhodesian species is distinct from the two Indian ones. Since then, *cuthbertsoni* has been sent me by Mr. Symes from Entebbe, and by Dr. Lamborn from Nyasaland. It is apparently a common species on the eastern side. As far as I know at present, the true *nebulo* F. is not found in the Ethiopian region. I am still in doubt whether the species noted as *nebulo* in the Palaearctic region is that species.

SORBENS GROUP

Musca sorbens Wiedemann. This species is widely distributed in the region, and is usually well represented in most collections. It is essentially a bazaar fly, and may commonly be seen sitting on food-stuffs of all kinds ; it rarely enters houses in the tropics. It is also commonly seen feeding on secretions from sores, wounds, etc., and particularly on the discharge from eyes. It is also attracted to sweat, and is a very annoying pest, alighting and crawling about on the skin. In view of these habits, it is an important carrier of pathogenic organisms, particularly of the bacteria which cause conjunctivitis and other diseased conditions of the eye. It breeds in horse, human and cow faeces, and especially in single deposits. The width of the ♂ vertex varies considerably (see Pt. 1) ; the colour of terga 1 and 2 also varies. For its synonomy

description and illustrations of the ♂ terminalia, see Pt. 1 ; for descriptions of adults and the illustration of the posterior spiracles of the third larva, see Pt. 2.

Musca vetustissima Walker. List of Diptera in the British Museum, IV, 902, 1849.

SYNONYMS: *humilis* auct. nec Wied.; *corvina* Froggart nec Fab.; *minor* Patton nec Macq. ; *niveisquama* Thomson ; *pumila* Macq.

MALE. HEAD. Eyes bare, with an area of slightly larger facets, as in *sorbens*. Vertex very narrow, almost linear, about one-tenth to even one-twelfth width of an eye. Lower vertex and cheeks satiny. MESONOTUM. Ground colour bluish-grey, with two broad black stripes. ABDOMEN. Dark orange, with silvery and dark markings ; terga 1 and 2 black ; tergum 3 orange, with a broad black median stripe extending slightly along anterior border, edged on each side with silvery stripes, varying in extent and usually well marked, edges of tergum silvery ; tergum 4 orange, with a narrower median black stripe similarly edged with broad silvery stripes, and with broad marginal silvery patches ; the lower border of the tergum usually has a narrow black band ; tergum 5 orange, with a median dark area which is silvery in some lights, and with marginal silvery patches.

MALE TERMINALIA. Fig. 3. ANAL CERCI. Fig. 3, *d*. Similar in shape to those of others of the group ; outer ends rounded, and inner ones not forming nipples. PHALLOSOME. Fig. 3, *a*. Chitinous portion long ; posterior process short and expanded at end (fig. 3, *c*), but not forked. PARAMERE. Fig. 3, *a*. Anterior part rather long, with six short bristles and one very long one about the middle ; posterior part forming a short cone. FIFTH STERNUM. Fig. 3, *e*. With characteristic dark areas on each side at distal end ; postero-lateral processes broad, long and as a rule well serrated.

FEMALE. HEAD. Vertex wide, about half width of an eye ; outer vertical bristles in two rows. MESONOTUM. Ground colour bluish-grey, with two broad black stripes divided in front of suture, Y-shaped. ABDOMEN. Bluish-grey, with black markings (chequered) ; terga 1 and 2 black ; tergum 3 steel-grey to bluish-grey, with a broad black median stripe, a broad black band along the posterior border, and black admedian stripes (these sometimes incomplete) ; tergum 4 very similar, but stripes narrower and either with or without a black band along posterior border ; tergum 5 mainly silvery, but with a narrow median black stripe.

EARLY STAGES. Oviparous, eggs laid in batches in animal excrement and also in human faeces. THIRD LARVA. Anterior spiracles with 8–10 finger-like processes. POSTERIOR SPIRACULAR PLATES. Fig. 3, *b*. Somewhat similar to those of *sorbens*, separated by about one-quarter width of a plate, the peritreme wide, and the breathing slits long and sinuous.

NOTES. *Musca vetustissima* is widely distributed in the region, and like its ally *sorbens* is essentially a bazaar fly ; it is also seen on and about animals and excrement. It is sometimes difficult to distinguish from *sorbens*, the main

and most reliable distinguishing characters being as follows :—The vertex of the ♂ is distinctly narrower than that of the ♂ *sorbens*, and is about one-ninth the width of an eye, whereas in the typical ♂ *sorbens* the vertex is about one-sixth to one-eighth eye-width. The mesonotum is much darker and has a bluish colour ; the abdomen is much darker orange, with well-marked silvery

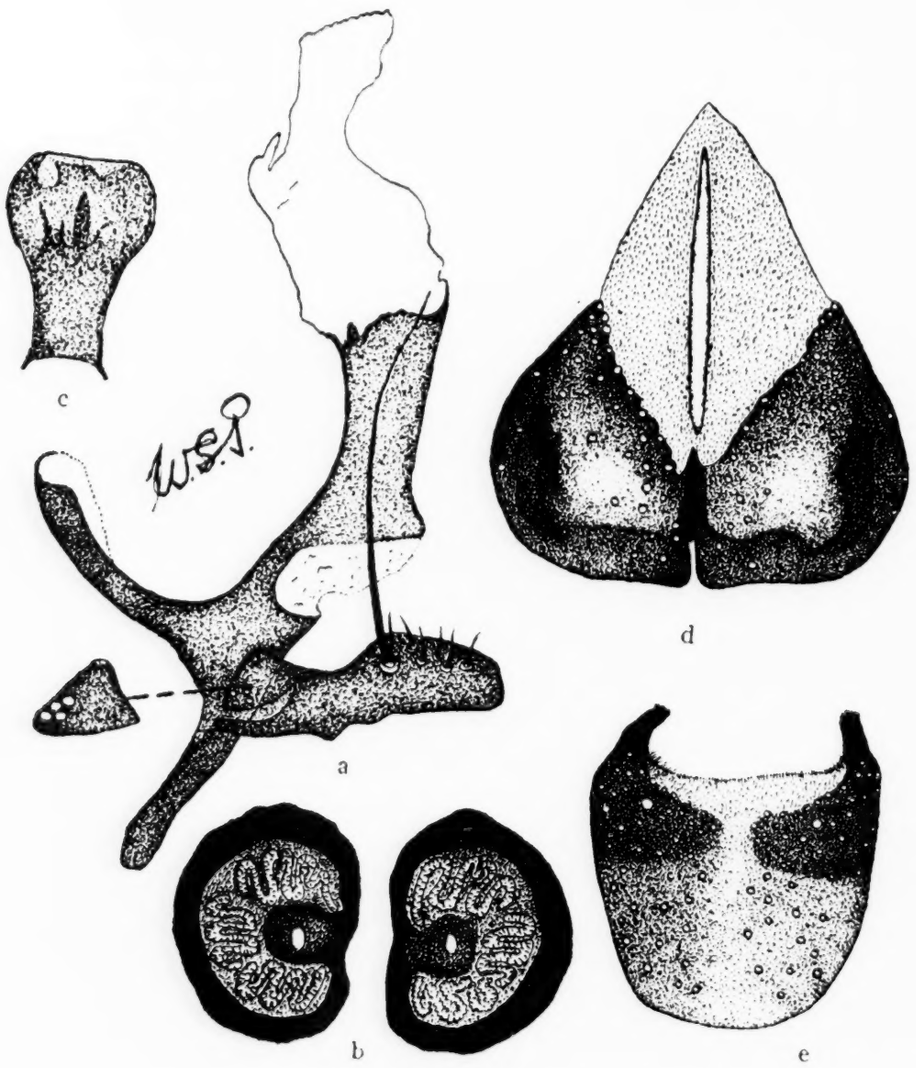


FIG. 3. *a.*—Phallosome and one paramere of *vetustissima* in side view ; *b.*—Posterior spiracles of third larva ; *c.*—Posterior process of phallosome, showing how it is expanded ; *d.*—Anal cerci ; *e.*—Fifth sternum.

patches ; the median stripe on terga 3 and 4 is usually wider than in *sorbens*. The vertex in the ♀ is about half eye-width, whereas in the ♀ *sorbens* it is usually about three-quarters.

Musca interrupta Walker. Dipt. Saund., 343, 1853.

SYNONYM : *lasiopthalma* Thomson.

MALE. HEAD. Eyes with numerous long hairs. Vertex very narrow, about one-eighth to one-ninth width of an eye ; proboscis long and slender, especially the haustellum. MESONOTUM. Dark metallic green, without any markings ; presutural dorsocentrals wanting. Wing veins markedly yellow, especially at base. ABDOMEN. Light orange or yellow ; terga 1 and 2 orange, with a broad dark (metallic green) triangular area occupying the middle, the postero-lateral margins yellow ; tergum 3 orange, with a broad dark median

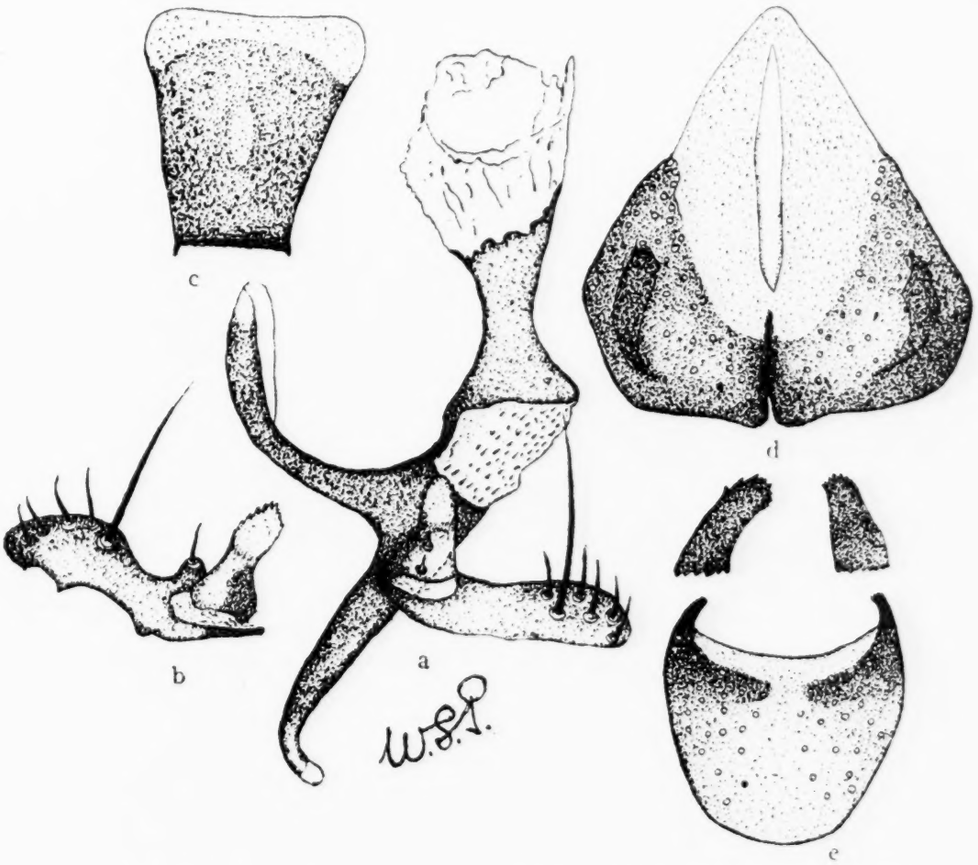


FIG. 4. *a.*—Phallosome and one paramere of *interrupta* in side view ; *b.*—One paramere ; note the elongated posterior part ; *c.*—Posterior process of phallosome, showing how it is expanded ; *d.*—Anal cerci ; *e.*—Fifth sternum.

stripe expanding T-shaped anteriorly ; tergum 4 with a large silvery patch in middle, sides orange, a suggestion of an interrupted narrow median dark stripe, forming spots anteriorly and posteriorly ; tergum 5 almost entirely silvery, with metallic sheen and sometimes with dark spots at posterior margin.

MALE TERMINALIA. Fig. 4. ANAL CERCI. Fig. 4, *d.* Free margin not quite so straight as in the cerci of *vitripennis* (Pt. 1, p. 395); inner ends not forming nipples. PHALLOSOME. Fig, 4, *a.* Chitinous part rather short, and narrow

in the middle ; posterior process long, expanded at the end but not forked. PARAMERE. Fig. 4, *a*, *b*. Long and rather narrow when seen in side view, with one long and 4–6 shorter bristles ; posterior part cone-shaped, the end clear and often minutely serrated, with four short spines at base and often one on a raised area. FIFTH STERNUM. Fig. 4, *e*. Small, the postero-lateral processes long and serrated.

FEMALE. HEAD. Eyes minutely haired. Vertex grey, about the width of an eye ; vertical stripe a little more than half width of vertex ; outer vertical hairs small and arranged in three or four rows. MESONOTUM. Grey, with some metallic sheen and four broad (green) stripes ; presutural D.C. wanting, as in ♂ ; wings also as in ♂. ABDOMEN. Light grey with some yellowish sheen ; terga 1 and 2 black ; tergum 3 grey, with median dark stripe, also broad admedian

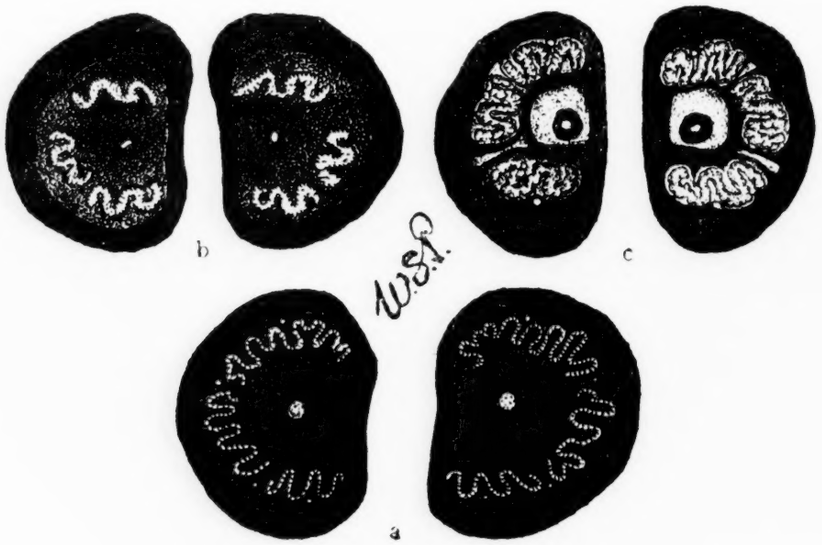


FIG. 5. *a*.—Posterior spiracles of third stage larva of *lusoria* ; *b*.—Same of third larva of *conducens* ; *c*.—Same of third larva of *interrupta*.

dark stripes and edges grey ; tergum 4 grey, with some dark stripes, as in tergum 3, only narrower ; or tergum may be almost entirely grey with suggestions of dark stripes, especially the median one ; tergum 5 almost entirely grey, with stripes as in tergum 4.

EARLY STAGES. Oviparous, eggs laid in batches in cow-dung but also in other excrement. THIRD LARVA. Anterior spiracles with 8–9 finger-like processes. POSTERIOR SPIRACULAR PLATES. Fig. 5, *c*. Small, long, with well-marked peritreme, separated by about one-fifth width of plate, breathing slits relatively short. Puparium dark brown.

NOTES. *Musca interrupta* is the common small haematophagous species of South and East Africa ; it also occures in the west. It is common on and about animals and excrement ; it may be seen feeding on cuts, wounds, sores and the discharge from eyes. It is closely allied to the European *vitripennis*.

In Pt. 2 it was noted that the eyes of the ♀ *vitripennis* are bare ; this, however, is an error, as they have microscopic hairs which are not so easily detected as are those of the ♀ *interrupta*. The early stages have been studied by Mr. Cuthbertson.

Musca tempestatum Bezzi. Ditt. Erit., II, 101, 152, 1908.

Male. Head. Eyes bare. Vertex silvery, narrow, about one-ninth to one-tenth eye-width. Proboscis with long labella (characteristic). Mesonotum. Grey, with two broad black stripes ; wings with yellow veins. Abdomen. Grey or bluish-grey ; terga 1 and 2 black ; tergum 3 grey, with broad black

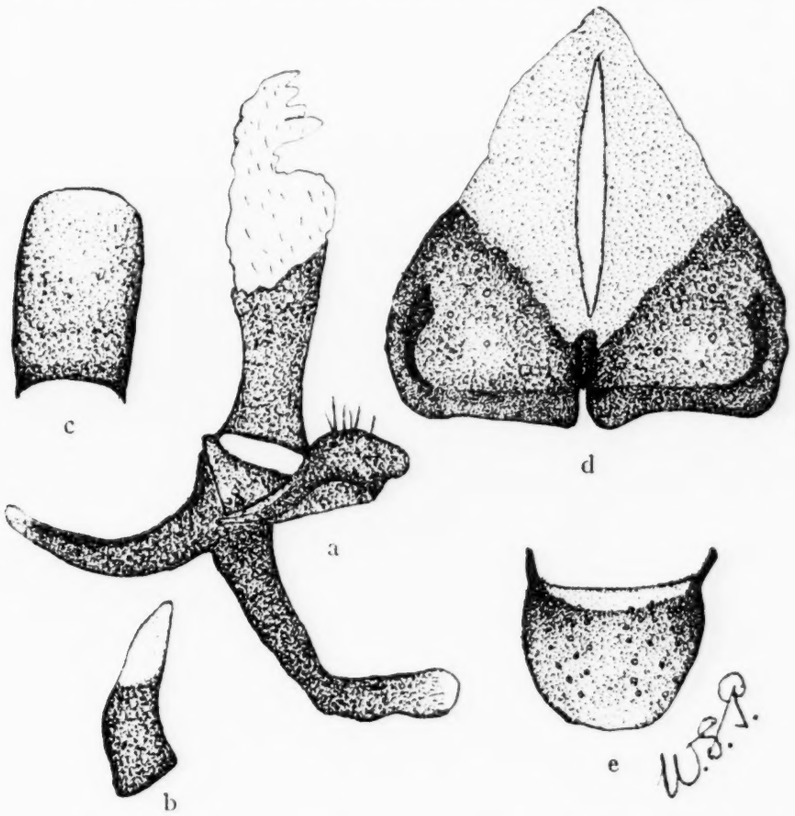


Fig. 6. *a*.—Phallosome and one paramere of *tempestatum* in side view ; *b*.—Posterior part of paramere ; *c*.—Posterior process of phallosome, showing how it is expanded ; *d*.—Anal cerci ; *e*.—Fifth sternum.

median stripe, which forms a complete dark band along posterior border, also a complete (sometimes incomplete) one along anterior border ; tergum 4 similar, the bands much narrower ; in some lights there is a dark band to the edge of tergum ; tergum 5 grey, with a very narrow median dark stripe.

Male Terminalia. Fig. 6. Anal Cerci. Fig. 6, *d*. Similar to those of others of the group, the posterior borders almost straight ; no nipples at inner ends. Phallosome. Fig. 6, *a*. Chitinous part rather narrow ; posterior process long and well expanded at end, but not forked. Paramere. Fig. 6, *a*, *b*. Anterior part long and rather narrow, bluntly pointed at end (side view) with

5–6 short bristles ; posterior part raised and bluntly pointed. FIFTH STERNUM. Fig. 6, *e*. Small, postero-lateral processes long and narrow.

FEMALE. HEAD. Vertex grey, wide, about equal to eye-width ; outer vertical hairs small and in two rows. Proboscis as in ♂, with very characteristic long labella. MESONOTUM. As in ♂. ABDOMEN. Grey ; terga 1 and 2 black, the posterior border sometimes grey; tergum 3 grey, with a broad black median stripe expanding anteriorly and posteriorly to form bands, as in ♂, dark narrow admedian stripes ; tergum 4 very similar to 3, except that stripes and bands are narrower ; tergum 5 grey, with an indistinct median black stripe.

EARLY STAGES. Unknown.

NOTES. *Musca tempestatum* was described by Bezzi from Abyssinia. It is widely distributed on the eastern side, extending down to Natal. It is haematophagous in habit, and is found on and about animals and excrement.

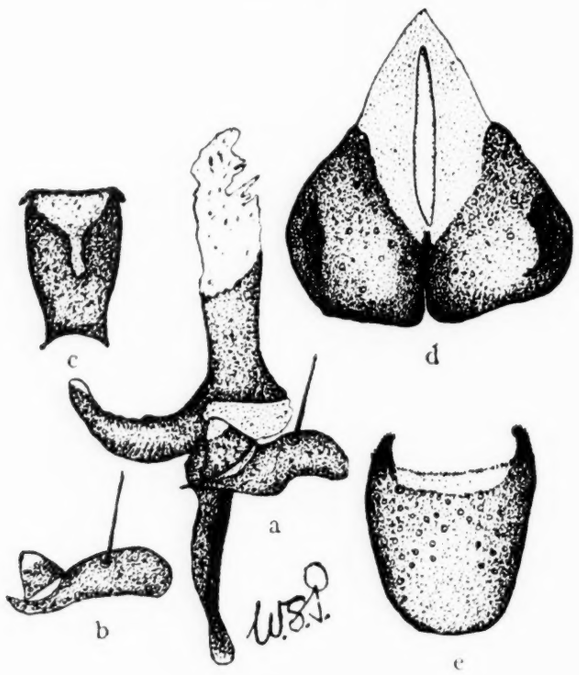


FIG. 7. *a*.—Phallosome and one paramere of *fasciata* in side view ; *b*.—Right paramere ; note the cone-shaped posterior part ; *c*.—Posterior process of phallosome, showing how it is expanded ; *d*.—Anal cerci ; *e*.—Fifth sternum.

Musca fasciata Stein. Proc. Linn. Soc. Lond., XIV, 149, 1910.

MALE. HEAD. Eyes bare. Vertex very narrow, linear ; cheeks silvery. MESONOTUM. Dark grey, with two broad black stripes, their anterior ends being sometimes separated by a narrow grey line. ABDOMEN. Grey, with black markings ; terga 1 and 2 black ; tergum 3 bluish-grey, with a broad black median stripe, a broad black admedian stripe on each side, the grey area appearing as a spot between black stripes and a black posterior band ; tergum 4 very similar, the median and admedian stripes narrower ; tergum 5 mainly bluish-grey, with a narrow incomplete median dark stripe and anterior dark band.

MALE TERMINALIA. Fig. 7. ANAL CERCI. Fig. 7, *d*. Very small, similar to those of the species of the group ; outer ends rounded, and inner ends not forming nipples. PHALLOSOME. Fig. 7, *a*. Chitinous part short ; posterior process also short, with the end expanded but not forked. PARAMERE. Fig. 7, *a*, *b*. Anterior part long and narrow (side view), with one stout bristle ; posterior part a small cone, the end clear. FIFTH STERNUM. Fig. 7, *e*. Small, dark, the postero-lateral processes short and well serrated.

FEMALE. HEAD. Vertex wide, grey, about three-quarters eye-width ; outer vertical hairs small and in a single row. MESONOTUM. Similar to that of ♂, but greyer, and the stripes commonly separated at the anterior end. ABDOMEN. Dark grey and very similar to that of the ♂, the admedian stripes being, if anything ,better marked.

EARLY STAGES. Unknown.

NOTES. This small dark species was described by Stein from the Seychelles. I have had a long series collected by Dr. Adams from cattle in Mauritius. It is also widely distributed on the eastern side of the Continent ; I do not know if it is found in Central and West Africa ; it is not common in collections. It is haematophagous in habit, and is found on and about animals and excrement. It is a troublesome fly, settling on the human skin in search of sweat.

Musca conducens Walker. Proc. Linn. Soc. Lond., IV, 138, 142, 1859.

SYNONYMS : *Pristirhynchomyia lineata* Brunetti ; *sorbens* Patton nec Wied.; *pulla* Bezzi.

MALE. HEAD. Eyes bare. Vertex very narrow, linear. MESONOTUM. Ground colour grey to dark grey, with some metallic sheen and with four black stripes, which often appear fused anteriorly. ABDOMEN. Light orange ; terga 1 and 2 either dark brown or black—if the former, the postero-lateral margins are orange ; tergum 3 light orange, with a narrow black median stripe extending along the anterior border to form a dark band ; tergum 4 similar, but median stripe narrower ; tergum 5 almost entirely silvery, with a faint dark median stripe and orange at edges. If the abdomen is viewed directly from above, terga 3–5 appear almost entirely silvery, owing to the silvery dusting.

MALE TERMINALIA. Illustrated in Pt. 1. ANAL CERCI. Outer ends markedly rounded ; inner not forming nipples. PHALLOSOME. Chitinous part rather slender ; posterior process long and expanded, but not forked at end. PARAMERE. Anterior part. Long and narrow (side view), with 3–6 short bristles, one commonly longer than the others ; posterior part a well-marked cone, or narrower with rounded end. FIFTH STERNUM. Small, dark at sides of distal end, postero-lateral processes long and slender.

FEMALE. HEAD. Vertex wide, about three-quarters width of an eye ; vertical stripe more than half width of vertex ; outer vertical hairs in a single row (with one or two additional hairs). MESONOTUM. Grey, with four black stripes never tending to unite. ABDOMEN. Grey, with black and orange

markings ; terga 1 and 2 dark brown to black ; tergum 3 grey, with olivaceous sheen, a narrow black median stripe expanding along posterior border to form a band ; tergum 4 similar, but with much narrower median dark stripe and posterior band ; tergum 5 mainly grey with silvery reflections, a suggestion of a median stripe.

EARLY STAGES. Oviparous, eggs laid in batches, mainly in patches of cow-dung but also in excrement of other animals. THIRD STAGE LARVA. Anterior spiracles with 7–8 finger-like processes. POSTERIOR SPIRACULAR PLATES. Fig. 5, *b*. Rather small, close together, and breathing slits relatively short.

NOTES. *Musca conducens* is widely distributed in the region. It is a haematophagous species and is commonly seen on and round animals in the field, especially cattle, feeding at sores, cuts and wounds. It has five quite well-developed prestomal teeth, which it can use to scratch off a scab or crust (see previous paper, 1933*a*).

Musca ventrosa Wiedemann. This species with its orange-coloured, unmarked abdomen is one of the easiest species to recognize. It is widely distributed in the region, but is not common in collections. It is haemato-phagous in habit, and is only seen about animals and excrement. For its synonomy, the description of adults and illustration of the ♂ terminalia, and that of the posterior spiracular plates of the the third larva, see in Pt. 2.

Musca albina Wiedemann. This characteristically coloured species without sternopleural bristles has been recorded from Dahomey and from South and South-West Africa. It is a desert or semi-desert species. The puparium is white. For its synonomy and description of adults, see Pt. 2 ; for illustrations of ♂ terminalia, see Pt. 1.

Musca crassirostris Stein. This well-known blood-sucking species is widely distributed in the region, and is a pest of cattle and horses. Du Toit and Nieschulz (1933) have recently recorded it from South Africa (Pretoria district). Its proboscis has been fully described by Cragg (1912), and the tearing apparatus has been illustrated by me in a recent paper (1933*a*). For its synonomy, description of adults and illustrations of the posterior spiracular plates of third larva, see Pt. 2 ; for illustrations of the ♂ terminalia, see Pt. 1.

Musca tempestiva Fallen. This small grey species is widely distributed in the region. It is only found on and about animals and excrement ; it is haematophagous in habit, and is a most annoying fly, settling on the human skin in search of moisture. For descriptions of the adults, illustrations of the ♂ terminalia and the posterior spiracles of the third larva, see Pt. 2.

LUSORIA GROUP

Musca lusoria Wiedemann. Auser. Zwei. Insekt., II, 411, 46, 1830.

Male. Head. Eyes bare. Vertex narrow, about one-tenth eye-width; cheeks silvery. Mesonotum. Grey, with four broad black stripes. R_{4+5} with a row of small hairs along ventral surface. Abdomen. Orange, with silvery and dark markings; terga 1 and 2 black; tergum 3 orange, with a broad black median stripe, silvery patch on each side, also at extreme edges, and sometimes a narrow dark band along posterior border; tergum 4 very similar, but median dark stripe narrower, silvery patches more extensive, and dark band at posterior border wider and always present; tergum 5 dark in the middle, with silvery dusting.

Male Terminalia. The male terminalia are illustrated and noted in Pt. 1. The anal cerci are produced at the outer ends into a broad process, and at their inner ends into a long narrower one; at present I know only one other species (*spinohumera* Awati) which has similar cerci. The phallosome has a long chitinous portion; the posterior process is wide and hood-like, and slightly forked at the end. The anterior paramere is long and wide, with 3–5 bristles, two usually long; the posterior part is long, bent upwards, and has several short spines on elevated areas. The fifth sternum is long, wide and dark at the sides, and the postero-lateral processes are short, wide and bent inwards.

Female. Head. Vertex wide, about equal to width of an eye; the outer vertical hairs are in four rows. Mesonotum. Grey, with four black stripes, as in the ♂. Abdomen. Grey, chequered; terga 1 and 2 black, with grey spots on each side of the middle line; tergum 3 grey, with a black median stripe, admedian black stripes expanding to form a narrow posterior band; intervening areas grey, edges silvery; tergum 4 very similar; tergum 5 silvery, the median part dark.

Early Stages. Larviparous, deposits one second stage larva at a time in cow-dung and in the faeces of other animals. Third Larva. Anterior spiracles with 6–8 finger-like processes. Posterior Spiracular Plates. Fig. 5, *a*. Large, wide, densely chitinized, breathing slits narrow, very long and sinuous.

Notes. *Musca lusoria* is the common large haematophagous species of the region and is found practically everywhere. It is common on and about animals, feeding on cuts, wounds, sores and especially on the discharge from eyes.

Musca xanthomelas Wiedemann. Auser. Zwei. Insekt., II, 54, 416, 1880.

Synonyms: *alba* Mall.; *albomaculata* Macq.; *dorsomaculata* Macq.; *rufiventris* Macq.; *convexifrons* auct. nec Thomson.

Musca xanthomelas is a common and widely distributed species in the region. For description of the adults, illustration and notes on the ♂ terminalia and the

posterior spiracles of the third larva, see Pt. 2. A comparative study of the adults and ♂ terminalia of what I take to be *albomaculata* from Mauritius leaves no doubt that it is identical with *xanthomelas* Wied. It should be noted, however, that the area of large facets in the ♂, which is characteristic of *xanthomelas*, is better marked in the specimens from Mauritius. I have also had similar specimens from Kenya bred by Mr. Symes. At first I was in doubt whether these specimens represented a distinct species or a local variety, but I have been unable to detect any constant differences in the ♂ terminalia. *Xanthomelas* is a haematophagous species and is found on and about animals and excrement. It is a difficult species to identify, the important points to note being : the large facetted area in the ♂ (which may be marked), the linear ♂ vertex, the silvery cheeks, the dark bluish thorax with four stripes (sometimes only narrowly separated, especially in the ♂), the orange abdomen, and the characteristic dark bands on terga 3 and 4 in the ♀. The puparium is white.

Musca gabonensis Macquart. Dipt. Exot., Suppl. V, 115, 20, 1855.

Synonyms : *ethiops* Stein ; *scatophaga* Malloch.

Male. Head. Eyes bare. Vertex black, narrow, about one-sixth to one-seventh eye-width ; cheeks silvery. Mesonotum. Dark, with two broad black stripes ; R_{4+5} with a row of small hairs along the ventral surface. Abdomen. Almost entirely dark orange ; terga 1 and 2 black or nearly so, part of posterior border orange ; tergum 3 orange, with a narrow incomplete dark anterior band, and sometimes a narrow median stripe ; terga 4 and 5 dark orange, the latter with an indistinct median dark spot and some silvery dusting.

Male Terminalia. The phallosome, etc., is illustrated in Pt. 1. Anal Cerci. Fig. 8, *a*. Large, dark, outer ends rounded, inner forming small nipples. Phallosome. (See Pt. 1.) Chitinous part very short ; posterior process expanded into a wide hood ; apodeme of phallosome very characteristic, long, wide, and with markings suggesting a lyre (see Pt. 1, fig. 26, *e*) ; Paramere. (See Pt. 1.). Anterior part short and wide in side view, usually with two hairs, one long ; posterior part long and bent, with several spines on raised areas. Fifth Sternum. Fig. 8, *b*. Long, wide, with black patches on each side of distal end, postero-lateral processes long, bent inwards, wide and serrated.

Female. Head. Vertex black, wide, almost width of an eye ; cheeks silvery ; outer vertical hairs in several rows. Mesonotum. Dark grey, with two broad black stripes, sometimes separated anterior to suture ; R_{4+5} with hairs as in ♂. Abdomen. Light orange ; terga 1 and 2 light orange, with a black area at middle of anterior border and a short black posterior band ; tergum 3 orange, with much silvery dusting, and with a wider black posterior band ; tergum 4 very similar, with a suggestion of a narrow dark median stripe and a wider posterior dark band ; tergum 5 orange darker in the middle at the end.

EARLY STAGES. Unknown.

NOTES. This large species, with its orange abdomen and two dark stripes, is as a rule easily recognized. It is widely distributed, and is usually found about and on animals.

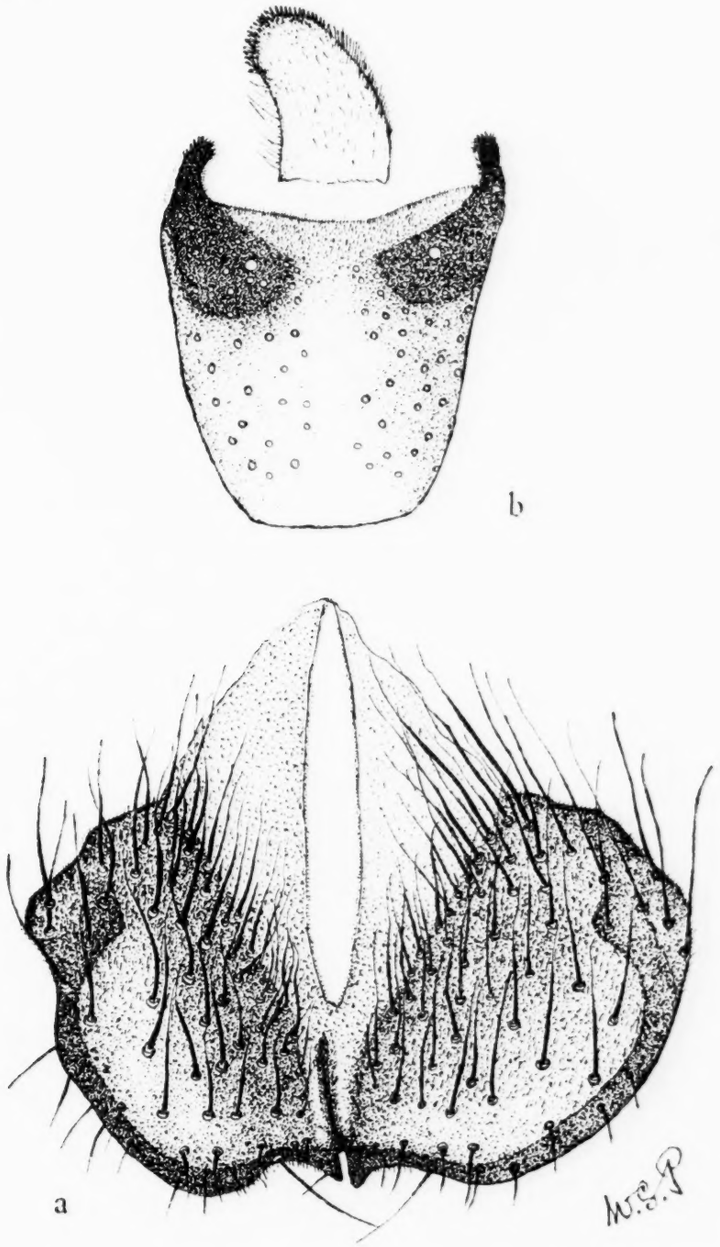


FIG. 8. *a.*—Anal cerci of *gabonensis* ; *b.*—Fifth sternum.

Musca natalensis Villeneuve. Ann. S. Afr. Mus., XV, 512, 1916.

MALE. HEAD. Eyes bare. Vertex narrow, about one-eighth eye-width. MESONOTUM. Grey, with two broad black stripes ; R_{1+3} with a row of small hairs along ventral surface. ABDOMEN. Light orange ; terga 1 and 2 orange,

a black patch at the middle of the anterior border, and a narrow median black stripe ; tergum 3 with a broader median dark stripe expanding along anterior border, well-marked silvery spots adjacent and at edges ; tergum 4 very similar, silvery patches better marked, and median dark stripe narrower and expanding to form a dark band along posterior border ; tergum 5 silvery in the middle, with a faint narrow median stripe, dark admedian ones and silvery edges.

MALE TERMINAL. Fig. 9. ANAL CERCI. Fig. 9, *e*. Large, dark, outer

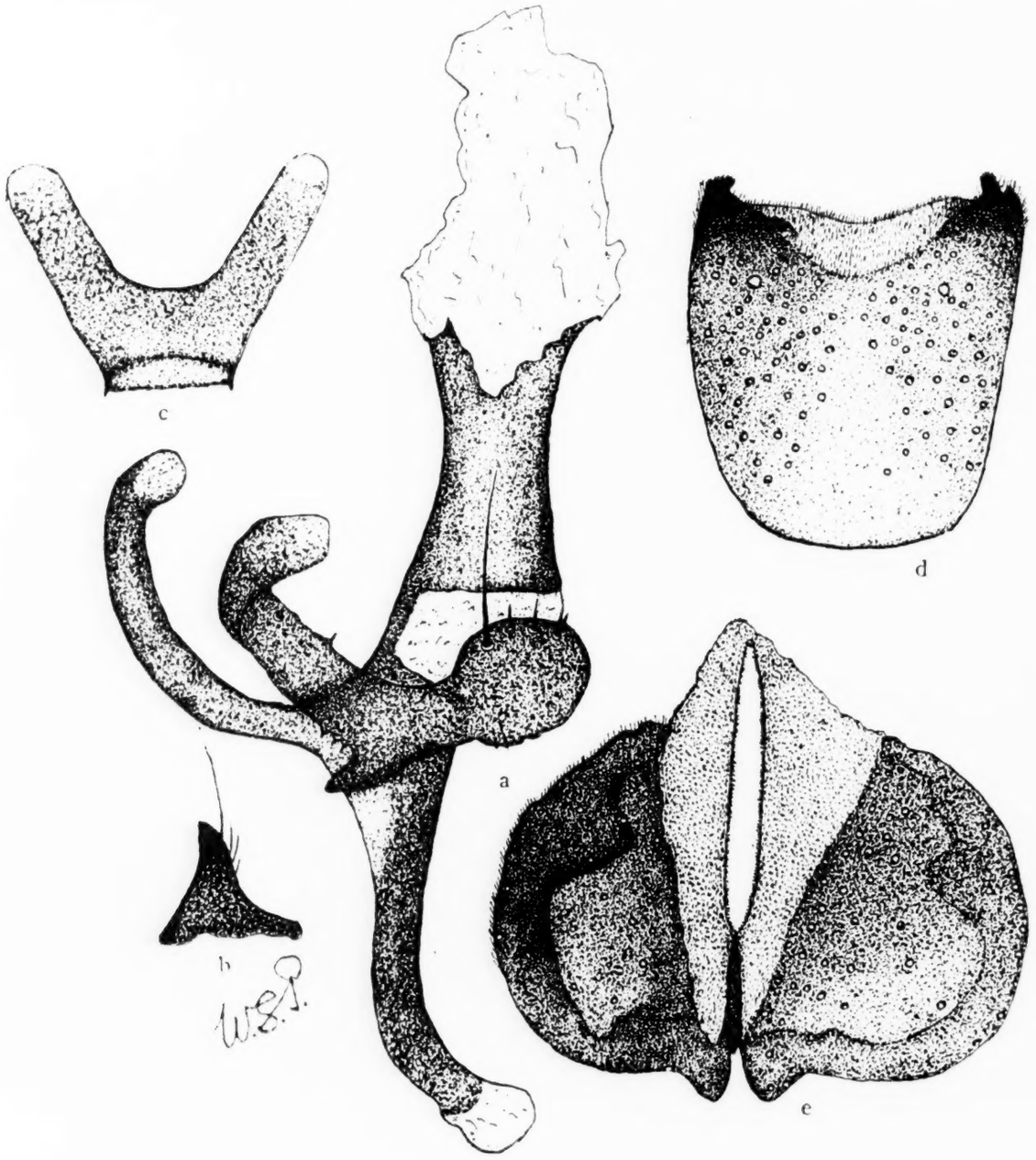


FIG. 9. *a*.—Phallosome and one paramere of *natalensis* in side view ; *b*.—Front view of anterior part of paramere ; *c*.—Posterior process of phallosome, showing how it is expanded and forked ; *d*.—Fifth sternum ; *e*.—Anal cerci.

ends rounded, the inner forming rather broad nipples. PHALLOSOME. Fig. 9, *a*. Chitinous part wide ; posterior process long, bent and deeply forked. PARAMERE. Fig. 9, *a*, *b*. Anterior part wide and rounded, with three small and one long bristle ; posterior part long, wide and bent upwards at end, with several spines. FIFTH STERNUM. Fig. 9, *d*. Long, wide, dark at distal ends, postero-lateral processes very short and bent inwards.

FEMALE. HEAD. Vertex wide, about equal to eye-width ; cheeks silvery ; outer vertical hairs in several rows. MESONOTUM. As in ♂ ; R_{4+5} also with hairs. ABDOMEN. Orange, with black and silvery markings ; terga 1 and 2 light orange, anterior border with a black spot, a very narrow median dark stripe ending in a dark incomplete band (the dark line joining the two dark areas is often wanting) ; tergum 3 orange, with a broad black median stripe expanding anteriorly and posteriorly to form bands, narrow admedian dark stripes, and silvery intermediate and marginal spots ; tergum 4 very similar, the median dark stripe broader ; tergum 5 with a dark median patch and silvery borders.

EARLY STAGES. Unknown.

NOTES. This large species with its two mesonotal stripes appears to be localized to Natal ; it was described by Villeneuve from Durban. It is probably haemotophagous in habit.

Musca dasyops Stein. Ann. Mus. Nat. Hung., XI, 468, 19, 1913.

MALE. HEAD. Eyes densely hairy. Vertex narrow. MESONOTUM. Black, with two broad black stripes. ABDOMEN. Black, with silvery patches ; terga 1 and 2 black ; tergum 3 silvery grey, with a broad black median stripe forming a broad black posterior band and a narrower one anteriorly, black admedian stripes, the intervening areas silvery grey ; tergum 4 similar, the stripes and bands narrower, and silvery areas more extensive ; tergum 5 also similar.

FEMALE. HEAD. Eyes densely hairy. Vertex wide, almost eye-width ; outer vertical hairs long and fine and in three rows. MESONOTUM. Similar to that of the ♂. ABDOMEN. Also similarly marked to that of the ♂.

EARLY STAGES. Unknown.

NOTES. This species was described by Stein from Kilama N'ajaro, and Villeneuve has also recorded it from the Aberdares. I have not had an opportunity of examining the ♂ terminalia, and I therefore only provisionally place it in this group. Nothing is known of its habits.

Musca alpesa Walker. List of Diptera in the British Museum, VI, 901, 1849.

SYNONYMS : *spectanda* auct. nec Wied.; *congolensis* Villeneuve.

MALE. HEAD. Eyes bare, with a rather well-marked large facetted area. Vertex very narrow, linear. MESONOTUM. Dark grey, with two broad black stripes ; R_{4+5} with a row of small hairs along the ventral surface. ABDOMEN. Orange ; terga 1 and 2 black ; tergum 3 orange, with a broad black median stripe, expanding T-shaped anteriorly, silvery patches on each side and at edges

of tergum ; tergum 4 orange, with a narrower median dark stripe and silvery patches, as in tergum 3, and with a well-marked posterior dark band ; tergum 5 silvery, with a median narrow dark stripe.

MALE TERMINALIA. Fig. 10. ANAL CERCI. Fig. 10, *b*. Large, the outer ends rounded ; the inner forming broad nipples. PHALLOSOME. Fig. 10. Chitinous part long and broad ; posterior process long, broad and expanded hood-like. PARAMERE. Fig. 10, *a*. Anterior part long, with one short and one

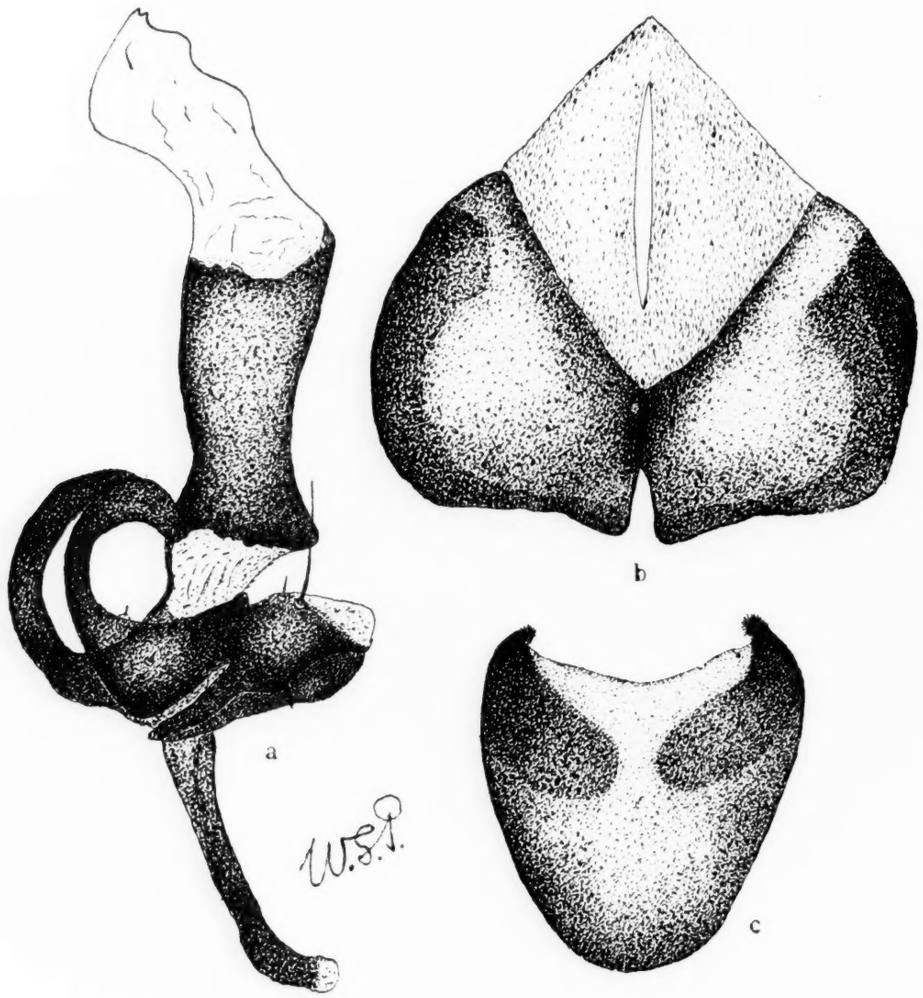


FIG. 10. *a*.—Phallosome and one paramere of *alpesa* in side view ; *b*.—Anal cerci ; *c*.—Fifth sternum.

long hair ; posterior part long, expanded and bent over at end. FIFTH STERNUM. Fig. 10, *c*. Long, dark at sides at distal end ; postero-lateral processes small, bent inwards and well serrated.

FEMALE. HEAD. Vertex wide, about three-quarters width of an eye ; outer vertical hairs in a single row below. MESONOTUM. Greyish-blue, with two broad black stripes separated Y-like anterior to suture. ABDOMEN. Orange ;

terga 1 and 2 dark brown to black, sometimes with two light spots on each side of middle line ; tergum 3 orange, with silvery dusting, a narrow median dark stripe, and a narrow dark posterior band ; tergum 4 similar ; tergum 5 silvery, with a dark patch in the centre.

EARLY STAGES. Unknown.

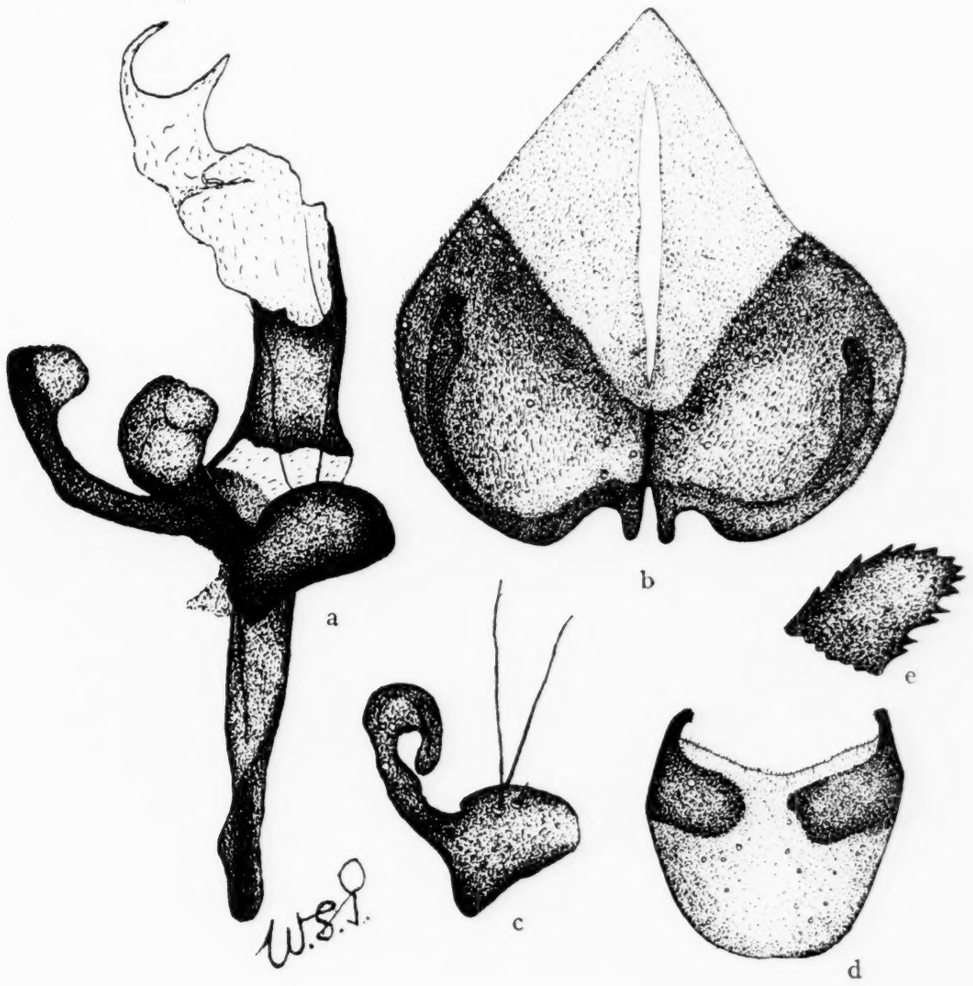


FIG. 11. *a.*—Phallosome and one paramere of *munroi* in side view ; *b.*—Anal cerci ; *c* —Right paramere ; *d.*—Fifth sternum ; *e.*—Posterior process of same.

NOTES. This large species was described by Walker from a single ♂ from Sierra Leone ; the ♀ was unknown to Walker. Mr. Gibbins collected two ♂♂ and one ♀ at Entebbe. As the ♂ agrees well with Walker's type, which I have examined, I believe that these specimens from Uganda are this species.

Musca munroi sp.nov.

MALE. HEAD. Eyes bare ; a well-marked large facetted area. Vertex linear. MESONOTUM. Grey, with two broad black stripes ; R_{1+5} with a row of

small hairs on ventral side. ABDOMEN. Orange; terga 1 and 2 dark brown to black; tergum 3 orange, with a dark median stripe expanding along anterior border to form a partial band; tergum 4 similar, with a narrow median black stripe; tergum 5 dark in the centre and yellowish at sides.

MALE TERMINALIA. Fig. 11. ANAL CERCI. Fig. 11, *b*. Outer sides rounded, and inner forming rather long narrow nipples, very like those of *xanthomelas*. PHALLOSOME. Fig. 11, *a*. Chitinous part short but wide; posterior process long and expanded hood-like. PARAMERE. Fig. 11, *a*, *c*. Anterior part broad and rounded, with one short and two long hairs; posterior part long, expanded at end, which is markedly bent over. FIFTH STERNUM. Fig. 11, *d*, *e*. Small, with dark patches at sides of posterior end; postero-lateral processes rather long, bent inwards and serrated.

FEMALE. EARLY STAGES. Unknown.

NOTES. Two ♂♂ of this rather small species from Prospect, Natal, were sent me by Mr. H. K. Munro. One of the specimens was labelled *Musca lucomelas* Wied., by C. H. Curran. It is necessary to point out, as long ago recorded by Stein, that *lucomelas* Wied. (the type of which I have seen) is *Graphomyia maculata*. I have much pleasure in naming this species after Mr. Munro. I have also had several ♂♂ bred from excrement by Mr. Symes; and some bred ♂♂ from Mr. Gibbins.

KEY TO THE ♂♂ OF THE ETHIOPIAN SPECIES BASED ON EXTERNAL CHARACTERS

1.	Vertex very wide, almost width of an eye	*domestica*
	Vertex never so wide, either linear or about one-third eye-width ...	2
2.	Species with metallic mesonotum and without dark stripes	3
	Species with non-metallic mesonotum and with dark stripes	5
3.	Eyes densely hairy	4
	Eyes bare	*tempestiva*
4.	Sternopleural bristles wanting	*albina*
	Sternopleural bristles present	*interrupta*
5.	Eyes densely hairy	*dasyops*
	Eyes bare	6
6.	Vein R_{4+5} with only a few hairs near the base on ventral surface, not extending to and beyond the radio-medial cross-vein	7
	Vein R_{4+5} with a row of small hairs on ventral surface, extending to and beyond the radio-medial cross-vein	16
7.	Species with four black stripes	8
	Species with only two stripes	13
8.	Species with abdomen entirely orange	*ventrosa*
	Species with abdomen either orange, olive or grey with dark bands and stripes	9
9.	Species of medium size, with olive abdomen and dark stripes; palps yellowish; mentum forming a characteristic shining bulb readily noted even when proboscis is withdrawn	*crassirostris*
	Medium-sized or small species; abdomen neither olive nor grey, but orange with dark markings; palps black; mentum not bulbous ...	10
10.	Medium-sized species with orange abdomen and dark markings; vertex either linear or about one-third width of an eye	11
	Small species with orange and silvery abdomen; vertex linear... ...	*conducens*

11. Large facetted area well marked ; vertex linear, thorax dark and abdomen orange with silvery markings ... ... *xanthomelas*
No marked large facetted area on eyes. Abdomen orange, with dark bands, stripes and silvery patches 12
12. Vertex one-third eye-width ... ... *vicina*
Vertex about one-seventh width of an eye ... ... *cuthbertsoni*
13. Presutural dorsocentral bristles wanting ; labella elongated ... ... *tempestatum*
Presutural dorsocentral bristles present ; labella normal in size ... 14
14. Small dark species ; abdomen grey, with extensive black bands and stripes ... ... *fasciata*
Larger species ; abdomen mainly orange, with dark bands, stripes and silvery patches 15
15. Mesonotum grey ; abdomen light orange ; vertex one-sixth to one-eighth width of an eye ... ... *sorbens*
Mesonotum bluish-grey ; abdomen dark orange ; vertex about one-tenth to one-twelfth eye-width ... ... *vetustissima*
16. Mesonotum with four black stripes ... ... *lusoria*
Mesonotum with two black stripes 17
17. Abdomen mainly dark orange, except terga 1, 2 and part of tergum 3, which has a faint black median stripe ... ... *gabonensis*
Abdomen lighter orange, with well-marked dark bands, stripes and silvery patches 18
18. Eyes well separated, vertex about one-eighth eye-width ... ... *natalensis*
Eyes close together, vertex practically linear 19
19. Eyes with a well-marked large facetted area ; a relatively small species. Anal cerci with narrow, rather long nipples on inner ends ... ... *munroi*
Eyes without a well-marked large facetted area ; a large species. Anal cerci with short, wide nipples on inner ends ... ... *alpesa*

Key to the ♀♀ of the Ethiopian Species based on External Characters

1. Species with sternopleural bristles wanting ... ... *albina*
Species with sternopleural bristles present 2
2. Species with unmarked orange abdomen ... ... *ventrosa*
Species in which abdomen is marked with dark stripes, bands and silvery patches 3
3. Species without presutural dorsocentral bristles ... ... *interrupta*
Species with presutural dorsocentral bristles 4
4. Eyes densely hairy ; very dark species ... ... *dasyops*
Eyes bare 5
5. Palps light orange ; mentum forming a characteristic shining bulb readily noted even when proboscis is withdrawn ... ... *crassirostris*
Palps dark grey to black ; mentum not forming a characteristic bulb ... 6
6. Vein R_{4+5} with only a few hairs near the base on ventral surface, not extending to radio-medial cross-vein 7
Vein R_{4+5} with a row of small hairs on ventral surface, extending to and beyond radio-medial cross-vein 16
7. Species with two broad black stripes 8
Species with four black stripes 11
8. Small grey species ; abdomen with dark bands and stripes 9
Medium-sized species ; abdomen orange, with dark bands, stripes and silvery patches 10
9. Haustellum elongated and labella long ... ... *tempestatum*
Haustellum and labella normal ... ... *fasciata*
10. Thorax light grey ; abdomen greyish-yellow to orange (if rubbed) ... *sorbens*
Thorax bluish-grey ; abdomen bluish-grey with black markings ... *vetustissima*

11. Medium-sized species ; either house- and bazaar-frequenting, or only found on and about animals and excrement 12
Much smaller species, only found on and about animals and excrement 15
12. Abdomen mainly bright orange and silvery 13
Abdomen with orange markings on terga 1-3, remainder dark with silvery dusting 14
13. Cheeks markedly silvery ; abdomen dark orange with silvery spots and well-marked dark posterior bands to terga ; only found on and about animals and excrement ; puparium white ... ... *xanthomelas*
Cheeks satiny ; abdomen bright orange with silvery patches and less-marked dark posterior bands to terga ; house- and bazaar-frequenting species ; puparium brown ... ... *cuthbertsoni*
14. Vertex almost equal to eye-width ; vertical stripe very wide, about three-quarters width of vertex, and with bulging sides ; terga 1 and 2 dark orange, remaining terga greyish with dark markings and some bluish sheen ... ... *domestica*
Vertex and vertical stripe very similar ; abdomen more extensively orange, especially terga 1 and 2... ... ... *vicina*
15. Mesonotum grey with four stripes not tending to fuse ; abdomen grey and black, with basal orange markings ; haustellum with mentum rather strongly chitinized, and prestomal teeth well developed (not well seen unless labella everted) ; a well-developed bristle about middle of posterior aspect of fore tibia ... ... *conducens*
Mesonotum yellowish-grey, the inner narrower stripes tending to fuse with outer behind suture ; abdomen mainly olive-grey ; haustellum normal and prestomal teeth not strongly developed ; no strong bristle about middle of posterior aspect of fore tibia. A much smaller species *tempestiva*
16. Large species, with four black mesonotal stripes ; abdomen black and grey (chequered) ... ... *lusoria*
Large species, with only two black mesonotal stripes 17
17. Abdomen mainly dark or light orange, dark markings much reduced ... *gabonensis*
Abdomen silvery and orange with dark stripes and bands 18
18. Terga 1 and 2 dark brown to black ; tergum 3 orange with silvery dusting, median dark stripe and posterior dark band ... ... *alpesa*
Terga 1 and 2 mainly light orange ; tergum 3 more orange, with median stripe and dark band ... ... *natalensis*

It will be noted that the ♀ *munroi* is not included in the above key, as it is not known.

In my next paper in this series I shall describe the Oriental species.

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STUDIES IN THE CHEMOTHERAPY OF MALARIA

THE INHIBITORY ACTION OF ANTI-MALARIAL DRUGS ON BLOOD LIPASES

BY

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(*Received for publication 22 October*, 1936)

Numerous theories have been put forward to explain the action of quinine in malaria. It has, for example, been suggested that the drug penetrates the red cells and kills the trophozoites by acting directly on the parasite. Morgenroth (1918) believes that when the drug is absorbed on the red cell surface the entry of malarial parasites to the cells is prevented. Another view is that the blood may be rendered unsuitable for the multiplication of parasites, and that they fall a prey to the various defence mechanisms of the body, which in the view of Yorke and Macfie (1924) include the production of immune body by the host, the parasites destroyed by the drug acting as suitable antigen. James (1929) is of the opinion that continued dosage by quinine has an adverse effect on the production of this immune body. The evidence for immune body, however, is still lacking. Kirschbaum (1923), who incubated equal amounts of blood containing *P. vivax* and 1 : 5,000 quinine at 38°, found that morphologically parasites were little affected, and his evidence is taken in favour of the view that quinine in unaltered form does not act upon the parasites. Bass (1922), on the other hand, also by *in vitro* experiments, brings forward evidence that the action is direct, as degeneration and death of the parasites was noted in the case of *P. falciparum*. Lourie (1934), when studying the effect of intraperitoneal injection of quinine in canaries infected with *P. cathemerium*, concluded that the therapeutic effects of quinine are not attributable to increase in the defence mechanism of the host, but to a direct action by quinine or metabolite on the parasite itself, causing a marked inhibition of growth and reproduction.

Quinine causes disintegration of *Paramoecium* in a concentration of 1 : 20,000, and of amoebae in greater dilution. On the other hand, salt-water amoebae and the spirochaete of relapsing fever can live in concentrations of 1 : 500. Purely chemical reactions between drugs and cells are not frequent, and physico-chemical relationships must be considered when we seek an explanation of drug action. The theories of lipoid solubility (Meyer-Overton), surface tension (Traube), absorption (Warburg) and permeability (Höber) have all been suggested in turn. Rosemund (1935) considers that in organic compounds the electrical characters of the drug are of importance, just as ions are in inorganic chemistry. He has studied the dipole moments of phenol-lactones with regard to anthelminthic properties, and claims that such measurements allow

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physico-chemical properties to be predicted. It seems to us, however, that the dipole moment of a substance is made up of many unknown factors, and that the experimental difficulties of determination are many.

Amongst other properties, quinine has long been known to inhibit enzymes of varied nature, and of interest is the inhibition of oxydase, which is responsible for the guaiac blue reaction.

With a view to obtaining information as to how a drug acts, an investigation of the influence of various anti-malarial drugs on different enzyme systems is being undertaken in collaboration with Sir S. R. Christophers. In the present communication are recorded the results of a study of the inhibitory action of some twenty pure drugs of known constitution and activity on the lipases of rabbit, monkey and human serum.

The drop method suggested by Rona and Michaelis (1911) was employed, using tributyrin, which lowers the surface tension of water considerably, as substrate. The hydrolytic power of the lipase for tributyrin is measured by the change in surface tension as indicated by the change in drop-number, while solutions are kept at the same temperature. The products of reaction are small in amount and do not appreciably affect the results.

The general procedure for the quantitative study of inhibitory action of lipases by drugs was as follows. At room-temperature, approximately constant, 1 c.cm. of serum, suitably diluted with distilled water, was mixed with various dilutions of drug in the form of salt—generally hydrochloride, although it has been shown that the anion is of no importance—and of known volume. Three c.cm. of a phosphate buffer (pH 7·4) prepared from acid potassium phosphate 1 part, and di-sodium hydrogen phosphate 10 parts, in M/3 concentration, was then added, and the mixture allowed to stand for 40–60 minutes. Occasionally precipitation of the drug was caused by addition of buffer, and in these cases the buffer was added after the tributyrin. Thirty c.cm. of tributyrin freshly prepared was then added. This is done by shaking 10 drops of the ester with 1 litre of distilled water for 1–2 hours, and, after allowing to stand for some hours in a separating funnel, drops floating on the surface can be separated. Filtration is carried out through a moist filter, and the first part is rejected. The filtrate is clear, and should not be used after the second day. In absence of other criteria of purity, the sample is considered satisfactory when 5–10 gm., on shaking with 200 c.cm. water, give a solution with the same drop-number as 3 drops of the ester in the same quantity of water under the same conditions. A special pipette is used to estimate the drop-number. It is approximately 16 cm. long, with a bulb of 2 cm. diameter and ground end. There is a graduation mark on the capillary stem above and below the bulb. The drop-number of the pipette is determined for pure water, pure tributyrin and for suitable mixtures of these, and the results are graphed. The amount of unhydrolyzed ester in a solution can thus be determined. In the case of a substance which lowers surface tension considerably, a series of curves for

different dilutions must be made. A drop-number of 90 and 140 for water and tributyrin respectively is suitable. The drop-count, if made directly, is apt to be tiring. A tile with a suitable number of squares (Bien) adjusted by hand for the drops to fall on to makes counting easier. We have devised an electrical counter* as follows (fig. 1) :—

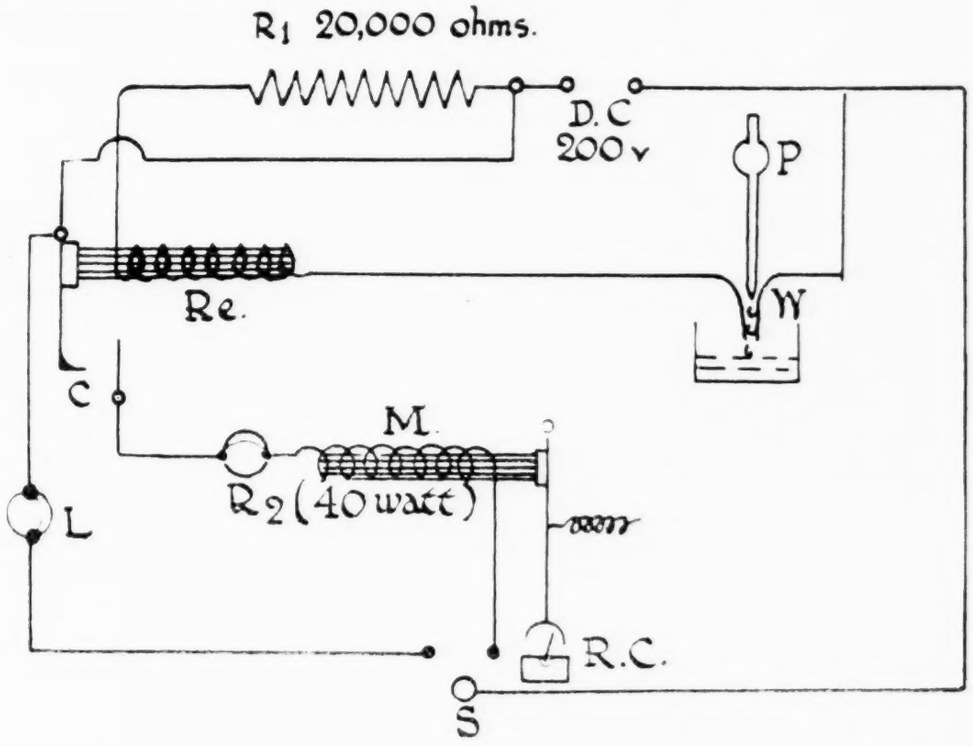


Fig. 1

The pipette P, filled above the mark, is put into a special holder which ensures that the tip always takes up the same position. The switch S is meanwhile turned to the left, and in that position a reading-lamp in the counting-box is lit. The meniscus of the fluid is adjusted to the mark on the pipette by means of filter-paper, and switch S is then turned to the right before a drop falls. As each drop falls, it bridges the gap between the wires W, causing a current to flow through the resistance R, and actuates the relay Re wound to work at 6 amps. This closes the contact C, sending current through the resistance R_2 and operating the electro-magnet M. The magnet pulls over an arm which moves the lever of a small rachet counter R.C. As the drop falls off the longer wire, the relay circuit opens and the arm of the electro-magnet is returned by a spring. The wires W are often a source of trouble, for unless accurately adjusted a drop may remain bridging the gap between them.

When the liquid in the pipette reaches the lower meniscus, the number on the counter is noted. Lamp L is lit in the counting-box, with switch S to the left, and so long as this is the case no drops are recorded during adjustment.

* I am indebted to Dr. A. R. Martin for much valuable help in its construction.

The formula by which K is calculated is that for a monomolecular equation, which Rona (1911) has shown to hold for the hydrolysis in question. By *h* is represented the coefficient of inhibition of the drug. When values of *h* are compared for similar concentrations of different drugs, under comparable conditions, we have a measure of the relative inhibitory powers of the drugs in question. The value Ko used in the calculation is the value of K when drug is absent, and Ka that when amount *a* of drug is present.

For the sake of clarity, an example of the inhibition of the ester-hydrolyzing power of rabbit, monkey and human serum lipase will be given in detail for one drug.

I. One c.cm. of rabbit serum 1 : 10 in distilled water was mixed with 3 c.cm. of isobutyldihydrocupreine hydrocholoride, and left 40–60 minutes. Thirty c.cm. of saturated tributyrin were then added, followed by 3 c.cm. of phosphate buffer pH 7·4. If, as was done in most cases, the buffer is added before the tributyrin, precipitation occurs. Temperature 18°–19°.

TABLE I

Drug in mgm.	Percentage of tributyrin after					$K = \frac{1}{\cdot 4343\ t} \cdot \text{Log}\ \frac{a}{a - x}$	$h = \frac{Ko - Ka}{Ko}$
	0′	30′	60′	90′	120′		
0	100	57	30	10	4	0·02252	
20	,,	88	79	—	79	0·00330	0·85
10	,,	85	79	66	60	0·00447	0·80
5	,,	88	68	54	40	0·00623	0·72
2·5	,,	77	59	47	36	0·00847	0·62
1·0	,,	68	49	35	24	0·01188	0·47
0·5	,,	66	46	35	20	0·01267	0·43
0·1	,,	64	39	20	10	0·01669	0·26

II. The conditions are the same as in experiment 1, but monkey serum 1 : 4 in distilled water was used.

TABLE II

Drug in mgm.	Percentage of tributyrin after						K	*h*
	0′	30′	60′	90′	120′	150′		
0	100	69	54	38	27	15	0·01127	
0·1	,,	—	—	—	—	87	0·00098	0·91
0·01	,,	92	87	—	82	79	0·00204	0·82
0·005	,,	92	82	75	72	66	0·00292	0·74
0·001	,,	79	66	54	48	41	0·00665	0·41
0·0005	,,	75	59	—	35	27	0·00882	0·22
0·00001	,,	75	56	—	31	18	0·00996	0·16

III. In this case human serum 1 : 1 in distilled water was used.

TABLE III

Drug in mgm.	Percentage of tributyrin after			K	h
	0′	75′	150′		
0	100	33		0·01487	
0·02	„		70·5	0·00233	0·842
0·0066	„		53·5	0·00417	0·718
0·0022	„		30	0·00803	0·457

From the results quoted in detail, the great difference in susceptibility of the lipases from various sources will be noted. Thus, in the case of rabbit serum, although more dilute, a concentration of drug which has little effect almost completely inhibits the monkey lipase and is far beyond the concentration used with human lipase. This is a general finding. Platt and Dawson (1925) have drawn attention to the fact that the ester-hydrolyzing power of pancreatic lipase is altered by the nature of the accompanying protein. They used olive oil and ethyl butyrate as substrates, and the added proteins were respectively edestin and albumin. To quote the authors : ' Clearly from these results, the same enzyme, associated with albumin, hydrolyses a simple ester such as ethyl butyrate more easily than a typical glyceride olive oil, which however is decomposed to a greater extent than ethyl butyrate when the lipase is allowed to work in association with edestin.' Falk (1924) had previously emphasized the importance of the protein structure of the lipase itself. The physico-chemical properties of the proteins present seem to us to be of importance. The question whether the drug is adsorbed by the lipase or enters into combination with it has been discussed by Stedman and Stedman (1931), who are of the opinion that both processes play a part ; and support is gained for their view from the work of Murray and King (1930), who have shown that optically isomeric alcohols inhibit liver esterase to different degrees, whereas simple adsorption ought to be equal for both.

Some facts relative to the question will be mentioned in a subsequent communication, when the distribution of drugs between red cells and serum in defibrinated blood will be discussed. Rona and Bach (1920), whose method we have used, found that for atoxyl and quinine there is an arithmetical decrease in the value of K, with geometric increase in the amount of drug added, and from this fact have developed a number of mathematical relationships. We did not find that this relationship held in many instances throughout the range of dilutions examined, but that after a time there was generally an unexplained change in the value of K through the series. If the value of K is plotted against

the logarithm of the drug concentration, taking Co (the concentration of drug which no longer inhibits enzyme action) as unity, the range of action between Co and Ct (the concentration of drug giving complete inhibition) is readily shown. The results for quinine on human lipase, where a linear relation holds, indicating agreement with the above authors, are shown in fig. 2. The

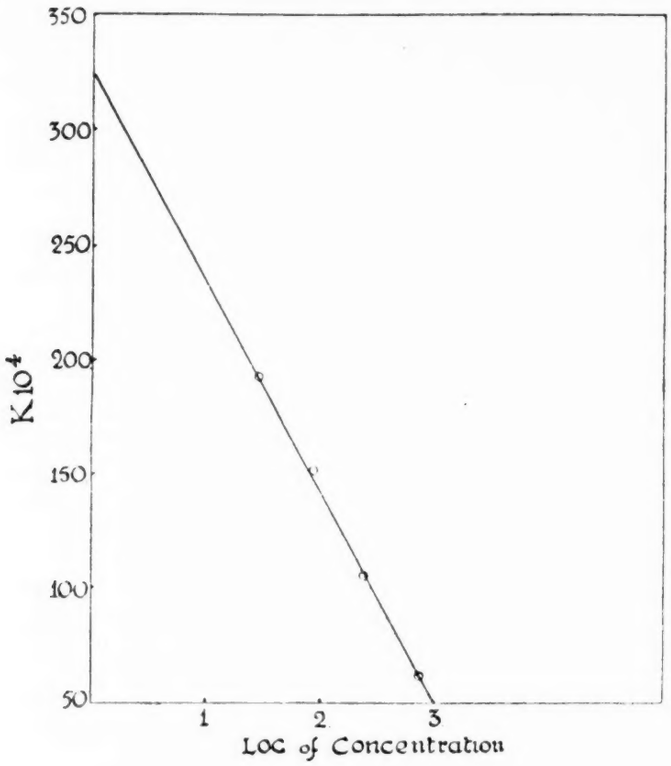


FIG. 2

solution was made up as follows :—

30 c.cm. tributyrin.
3 c.cm. phosphate buffer, pH 7·4.
0·333 mgm. quinine in 1 c.cm.
1 c.cm. human serum 1 : 1.
Temperature 19°.

TABLE IV

	Quinine in mgm.	K	Log. of concentration, with Co as unity
1	0	0·02145	
2	0·0012	0·01929	1·43
3	0·037	0·01512	1·92
4	0·111	0·01052	2·40
5	0·333	0·00627	2·87

The following are the values of h obtained with the different sera, when compared under similar conditions :—

TABLE V

	Rabbit serum	h for 10 mgm. drug in 37 c.cm.	Sign of rotation
1	Sodium atoxyl (sodium salt of p-aminophenyl arsinic acid)	0·940	
2	Vuzin (iso-octyl hydrocupreine)	0·883	
3	Dihydrocinchonine	0·845	D
4	Isobutyldihydrocupreine	0·802	
5	Cinchonine	0·771	D
6	Eucupin (iso-amyl dihydrocupreine)	0·770	
7	Cupreine	0·770	L
8	R 34 (8-β-aminoethylamino-6 methoxy quinoline dihydrochloride)	0·728	
9	Dihydroquinidine	0·671	D
10	Dihydroquinine	0·656	L
11	Dihydrocinchonidine	0·652	L
12	Cinchonidine	0·645	L
13	Quinine	0·615	L
14	Quinidine	0·607	D
15	Sodium stovarsol (sodium salt of 3 acetylamino-4-hydroxy phenyl arsinic acid)	0·589	
16	Optochin (ethyldihydrocupreine)	0·475	
17	Hydrocupreine	0·442	L
18	R 36 (8-γ-aminopropylamino-6 methoxy quinoline dihydrochloride)	0·422	
19	Plasmoquin	0·208	
20	Sodium quitenate	0·119	

TABLE VI

	Monkey serum	*h* for drug in 35 c.cm.			
		0·1 mgm.	0·5 mgm.	2·5 mgm.	Sign of rotation
1	Eucupin	0·953			
2	Vuzin	0·928			
3	Isobutyldihydrocupreine ...	0·913			
4	Optochin	0·874			
5	Atebrin		0·906		
6	Plasmoquin		0·904		
7	Hydroquinidine		0·864		D
8	Quinidine		0·714		D
9	R 34		0·701		
10	Hydrocupreine		0·674		L
11	Hydroquinine		0·670		L
12	Hydrocinchonine		0·661		D
13	Cinchonine		0·654		D
14	Quinine		0·640		L
15	Cinchonidine		0·635		L
16	Cupreine		0·624		L
17	Hydrocinchonidine		0·596		L
18	R 36		0·548		
19	Atoxyl			0·745	
20	Stovarsol			0·298	
21	Sodium quitenate			0·024	

Nos. 1–4 are not comparable with nos. 5–18, but it is evident that the order is correct for *h*, as 1–4 are more strongly inhibitory even in greater dilution. Atoxyl and stovarsol were not active inhibitors in the concentration used for the others, and should follow 1–18, as shown.

TABLE VII

	Human serum	*h* for drug in 36 c.cm.				
		0·01 mgm.	0·02 mgm.	0·2 mgm.	0·333 mgm.	Sign of rotation
1	Vuzin	0·926				
2	Eucupin	0·740				
3	Isobutyldihydrocupreine		0·842			
4	Dihydroquinidine ...			1·0		D
5	Quinidine			0·962		D
6	Optochin...			0·957		
7	Dihydroquinine ...			0·928		L
8	Plasmoquin			0·918		
9	Cinchonine			0·874		D
10	Dihydrocinchonine ...			0·799		D
11	Atebrin			0·796		
12	Quinine			0·697		L
13	Dihydrocinchonidine ...			0·693		L
14	Dihydrocupreine ...			0·682		L
15	Cinchonidine			0·553		L
16	R 36			0·440		
17	Atoxyl				0·753	
18	Cupreine				0·720	L
19	Stovarsol				0·120	

SUMMARY

The results quoted show that the inhibition of enzymes by definite amounts of drug under standard conditions may be followed quantitatively. Values for the inhibitory power are given by *h*, and this allows a comparison to be made between the different drugs. There is increased inhibition with quantity of drug added. We have also carried out experiments where the pH of the solution was not controlled; the method is not satisfactory. A constant value for pH is important in the case of salts, with regard to dissociation to base.

We see that the higher homologues of quinine, where the methyl of the methoxy group has been replaced by longer side chains (optochin, isobutyldihydrocupreine, eucupin, vuzin), are the most active inhibitors. This is in agreement with the findings of Acton (1922), who also states that the dextrorotatory quinine series are more strongly inhibitory than the laevo, and with this we partly agree. It is especially true for human lipase. In some cases the hydro alkaloids are more strongly inhibitory than the natural alkaloids. It may be that in certain cases combination between esterase and inhibitor takes place. This great inhibitory power of the drugs with long side chains is associated with a low partition ratio between red cells and serum, and the haemolyzing power of

the same drugs for red cells is reduced by the presence of serum. Those drugs which are active in malaria occupy an intermediate position in the tables. All the drugs employed contain basic groups, but little can be said of the variation of inhibitory power with structure. There is a marked difference in the relative and absolute sensitivity in the sera of different origin to any drug. The same inhibitory effect on human lipase is always obtained by a smaller quantity of drug than that for lipase of animal origin, and sometimes the difference in the amount of drug required is very large.

ACKNOWLEDGEMENTS. My best thanks are due to Sir Rickard Christophers for much help and encouragement in this work. I am indebted to Howards and Sons, Ltd., and to Bayer Products, Ltd., for the gift of drugs.

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STUDIES IN RURAL HYGIENE IN THE TROPICS

BY

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III.—SIMPLE FORMS OF SUBSURFACE DRAINAGE FOR ANTI-MALARIA WORK

(*Received for publication* 24 *October*, 1936)

The inhabitants of the great majority of districts in the rural areas of tropical and subtropical countries cannot afford to employ in sanitation certain methods which are stereotyped in urban conditions. One such method is that of the subsurface drainage of water in pipes as a means of preventing anopheline breeding. The economic condition of the peasants precludes their using for this purpose pipes made of metal or concrete, or even the common agricultural tile pipe. Not only is the initial cost of such piping high in most tropical rural areas, but the degree of erosion of metal and concrete pipes when laid down in certain soils is so great that frequent replacement is necessary.

Any form of instruction which lays claims to be regarded as at all suitable for peasants and villagers in the tropics ought to include a thorough grounding in elementary personal, domestic and local hygiene. Only by means of such education will the people develop any intelligent desire to improve the conditions which bring ill-health upon them, or to take measures to protect themselves. Since any measures taken by these impecunious villagers must of necessity be simple and inexpensive, all must realize that they have not now, and cannot for a long time hope to have, any means of purchasing supplies of such things as expensive pipes for anti-malaria work. Indeed, it will be very fortunate for them if, within a reasonable time, they can afford to obtain sufficient piping to bring to their doors clean and safe drinking-water. Therefore, if they wish to get rid of mosquito-breeding water by drainage, they will be compelled to devise methods which require only materials which can be procured locally. Experiments in adapting such local materials to the varied needs of rural sanitation may well lead to results which will compare not unfavourably with those obtained from the use of expensive manufactured articles by government departments, municipalities and large employers of labour, who alone can afford them. The present high cost of such materials may thus in the long run prove to have some compensation, since it will encourage the peasants to use local substitutes.

It is proposed here to give a brief account of a few of the simpler methods which are now undergoing trial as anti-malaria measures in certain parts of the East.

Stone drains. The first to which reference will be made are those in which stone is employed for drain-making. In several places in India and elsewhere

stone drainage has been used for anti-malaria work with a good measure of success, and in Malaya I saw some instances of its use on a fairly large scale. There it has been employed chiefly in certain *A. maculatus* areas, and has fulfilled surprisingly well its principal function, which is to lead water away from breeding places in such a manner that anophelines cannot use it for oviposition. This method can only be used in places where the ground to be drained is of suitable character. One condition essential before its adoption is the presence of a sufficiency of rock or local stone either on the site or within easy reach, so that the cost of transport can be avoided. Some advantage is gained if the material can be easily shaped into rough blocks of suitable size. Largely as a result of the existence in rocky ravines of the necessary conditions, streams there lend themselves to successful treatment, and the most advanced examples of it were demonstrated in such places. But given suitable ground, or a sufficient depth of existing watercourse, with adequate fall, and a good supply of stone in the vicinity, it appears to be a method the use of which could be widely extended in many places.

In one application of it, an earth drain is cut and left open for a time to harden, just as for laying down manufactured pipes in subsurface drainage. A layer of small stones is placed on the bottom of the graded trench, and on this layer a tunnel for the water is formed by aligning two rows of large stones and placing flat covering stones across them. Taking care to preserve the tunnel, large stones are then laid beside and over it, followed by smaller stones and finally gravel or porous soil up to the general ground level ; the surface of the finished drain may be planted with grass, if the depth of the drain is sufficient to eliminate the chance of blockage by the grass roots.

Another application is in ravines where the stream often runs in an irregular rocky bed. The water channel at the bottom of the ravine is roughly straightened, and several rows of large stones are laid and roofed with flat stones, as described above. The sides and top are carefully built up, and if, as is usual, there is plenty of depth, large stones can be used for building all the way up to the desired height ; the top layer is composed of large stones, as flat as possible, so as to make a good walking surface. In the neighbourhood of Penang, Dr. Eveson showed me some recent examples of the work done in various ravines.

Professor Williamson has come to the conclusion, as a result of observations and experiments, that anophelines will not penetrate down into the crevices between even large stones to any depth greater than about a foot or so. If the layer of closely packed stones overlying the water tunnel exceeds this in thickness, it is considered a fairly safe preventive against breeding. In most of the places seen, the stone packing overlying the water channel was several feet thick. The upper surface may be made sloping so as to follow parallel to the general fall in the bed of the stream, or it may be stepped down in terraces if this is found more convenient (Pl. XII, figs. 1 and 2). Even in situations where the layers of stones cannot be thick, the mosquito-breeding danger can be dealt with

successfully ; this is done by putting in as a mosquito-proof course a layer of fine stones or coarse gravel some little distance above the water channel. This layer is easily permeable to rain-water or even to any flood-water flowing over the surface of the stone packing, but does not permit the passage of mosquitoes. The flow of water from these stone drains is remarkably free, as can be observed at the outfall. It is possible in some cases, while standing on the flattened top of the finished stone packing, to hear the water running in the depth far below.

As regards cost, at one site which I examined it was stated that the cost of a cubic yard of stone-packed drain came to about four shillings, including blasting, breaking, transport and building up.

At the leper settlement on the island of Pulau Jerejerak some good examples of this type of drain were seen in ravines in the hill clearings. A very interesting and suggestive application of the method was demonstrated here by Dr. Eveson. At one point in the island an open concrete drain which had been laid some years ago had become badly eroded where the lengths of concrete were nearly touching each other. As a result, quite deep and sometimes wide pockets had formed in the exposed ground, and these remained full of seepage or overflow water from the drain itself, and *Anopheles maculatus* bred in them. The removal of the old concrete drain and the provision and laying of a new one in its place would have involved a good deal of expense. It was therefore decided to retain the old drain, utilizing the bottom of it as the water channel, and building over this a layer of stone packing, then putting a layer of gravel and finishing off the surface with stone to make it flat (Pl. XII, fig. 3) ; the result was said to be satisfactory in preventing the breeding of *A. maculatus*.

As regards the use of stone packing for drains, it was interesting to see what was practically this method in existence on the hill section of the Assam-Bengal Railway, where it was employed without any designs upon mosquitoes. From the summit of every rise there extended down in each direction alongside the railway a deep trench situated between the hill-side and the line ; presumably it had been originally a borrowpit for the construction of the railway track. This hollow had been filled up level with large stones, so that a wide and fairly regular pavement was formed for considerable distances ; on this stone-pack path gangers could walk alongside the line with comfort. Water coming off the hill was intercepted by it and percolated down through the interstices between the stones to a drain below ; in this it ran down-hill and was passed by culverts at suitable intervals under the railway to the lower side.

Bamboo drains. At Kuala Lumpur, Dr. Gross spent some time taking me round to see the use of bamboo as a substitute for manufactured pipes in subsoil drainage. Originally long lengths of bamboo were used, and these were converted into continuous pipes by cutting out a wedge-shaped piece at the position of each node and removing the septum. This system worked fairly well, but several objections to it were discovered as a result of experience,

Owing to the rough projections into the lumen of the pipe left when removing the septum, and owing to the aperture made in cutting out the septum, silting was facilitated ; even so, the long bamboos functioned fairly well in particular sites. The chief drawback to them, however, was that the cutting out of the septa so weakened the long pipes that when placed at any depth they gradually collapsed, and in consequence functioned imperfectly as drains.

A great improvement in the bamboo-drain method was effected by using the separate short lengths obtained by cutting across the bamboo close to each side of the septa. The resulting tubes, a foot or so in length, with clean-cut edges, are then used in exactly the same manner as if they were agricultural tile pipes. They are laid in the bottom of a properly graded and prepared trench, with a slight interval between their ends ; the junction is then covered with a thick crescentic pad of clay in such a manner that no water from above can enter between the adjacent ends of the pipes.

There are several points to be considered in employing this bamboo method : one is that some species of bamboo are more useful than others because they have a larger lumen in comparison with their outside diameter ; another is that experiment has proved that some kinds of bamboo are more resistant than others to the action of water and rotting. In all localities, therefore, even though there may be plenty of bamboo near at hand, better results will be obtained if the size of the lumen and the durability are studied. Nevertheless, it may still prove cheaper to transport suitable bamboo pipes from some distance than to put in any form of manufactured pipe, even taking into account the relatively shorter life of the bamboo drain. With regard to this last point, I was shown a sub-surface drain made in this material three years before. It was still discharging freely—quite as freely, I was told, as when it was first installed—and the condition of the bamboo appeared sound. The cost of a foot of bamboo pipe of 3 inches internal diameter works out locally at about one cent, as compared with 8–12 cents for agricultural tile pipes.

Percolation pits. This method of disposing of anopheline breeding water underground is being employed in selected places where the strata are suitable for it. Several good examples were shown to me in the flatter land at the foot of the Bhutan Hills, where Mr. Scott, manager of the New Dooars Tea Estate, has developed it greatly. It has been used here in order to get rid of man-made malaria, due to the piping of water to factories and lines. The overflow of tanks and waste water from taps trickling away in grassy hollows produced *A. minimus* breeding places in large numbers.

The method is a form of vertical drainage : a pit is dug down through the soil till a stratum of shingle is reached, and the water to be disposed of is led by a drain to the pit, from which it percolates away in the stone stratum. Some of the original pits first dug here were filled with large stones, but silting up soon occurred, and the stones were therefore removed and discarded. Then a rather more elaborate method was developed ; the pit was dug out in a rectangular

shape, and a solid concrete roof was built over it, provided with a mosquito-proof manhole for cleaning-out purposes ; in order to stop silt reaching the pits, ingenious silt-traps were placed on the course of the water inlet channel (Pl. XIII, fig. 1). The water may be put to various uses before entering the pit; for example, it has been used in some cases to fill drinking-troughs for numerous cattle. The silt-trap is accessible so that it can be cleaned easily as required ; hence it is only very rarely necessary to open the manhole and clean out the percolation pit itself.

A very important use of the method was demonstrated by Mr. Webb in natural breeding grounds on his estate. These were formed by grassy-bottomed stream-beds in fairly flat land ; these grass stream-beds become prolific breeding places after rains. In this region, pits dug through the soil to a depth of 3–8 feet reach a coarse gravelly stratum, and if a series of such pits is dug in the beds along the course of the streams the water percolates away. For a short period after heavy rains the pits themselves may be flooded, but the water quickly sinks and disappears (Pl. XIII, fig. 2). This is an easy and economical way of dealing with this type of breeding place in such suitable areas ; there are over 250 pits of this kind on the estate, and more are being made.

Stick and fascine drains. In this method, straight branches, either loose or tied in bundles, are employed ; the wood is not hollow, and the only drain channel provided for the water consists of the spaces which remain between the adjacent sticks. Dr. Waugh Scott, of Sungei Siput, showed me several examples of this kind of drain in all stages of construction. The graded and prepared trench being ready, bundles of straight branches about 6 feet in length and 2 inches in diameter are laid along the bottom of it (Pl. XIII, fig. 3). The thickness of the layer of sticks depends on the depth of the drain and on the amount of water to be disposed of. Over the stick bed a series of layers of lallang grass several inches thick is laid, each layer overlapping the next below it, like thatch on a roof. This grass layer is put down in order to prevent silt from the surface reaching the stick drain and so blocking up the interstices in it. Over the grass the trench is filled up with sand or the most porous soil which can be obtained locally, till it is almost level with the ground surface. Lallang grass is a long, tough and water-resistant grass which grows locally. Dr. Scott showed me how this grass is employed by tin-miners as a thatch and protection to prevent erosion by rain and flood of the bunds or dams, which they build to prevent silt from the dredger-workings reaching the rivers. It was said that such thatch, exposed to constant alternations of drying and wetting, had been found to remain effective for as long as ten years. It was this fact which suggested its use as a silt protection for stick drains.

In view of their cheapness and simplicity, a long life for drains of this type is not so essential ; but Dr. Waugh Scott informed me that drains originally put down by him a long time ago were still functioning well.

Herbage cover. When I was at Penang, Professor Williamson took me to see a great variety of expedients which he was trying for anti-malaria work. Quite the simplest of all these was the method of herbage cover. No doubt there are many modifications of it which will be improvized to suit local conditions and different sizes of breeding places.

The method was shown as applied to a length of a two-foot-deep grass-grown drain beside the road. Grass and weed and small branches of shrubs in the vicinity were cut by labourers, thrown into the water in the bottom of this ditch, and firmly pressed down ; further layers were added and trampled down until the ground level was reached.

The effects aimed at are partly mechanical prevention of mosquitoes from getting down to or coming up from the water, by the packing, and partly the production of chemical changes in the water itself, due to the vegetation gradually rotting ; these changes result in the production of a type of water in which most anophelines will not breed, although culicines may do so in large numbers. The use of bundles of vegetation for filling swampy areas, which is practised on many of the tea-gardens in Assam, has also the latter object, namely, to make the composition of the water unsuitable for anophelines.

In the case of larger streams with a more rapid run, where a rush of water may be expected after showers, it is necessary to support the herbage cover by putting in bars or stakes at intervals, so as to prevent the whole from being washed away. The method will no doubt be worked out on a larger scale by other observers whose work is in the rural areas, and it will be discovered whether it depends on the cover effect and the consequent subsurface draining away of the water, on the chemical modifications produced in the water during the decay of the herbage, or on other factors also.

I take the opportunity of drawing attention here to some experimental work already done on this method by Senior White (1936), an account of which appears in the last number of the *Records of the Malaria Survey of India* which I have just received. The conclusions are quite optimistic concerning the results obtained, as the penultimate paragraph shows ; ' Enough, I think, has already emerged to indicate its very great value in certain types of malaria protection work, and it will, generally it is hoped, and certainly within the writer's jurisdiction, be largely extended in the season 1936-37.'

REFERENCE

WHITE, R. SENIOR (1936). Williamson's ' herbage cover ' method of larval control : a preliminary note on some field trials. *Rec. Malar. Surv. India*, **6**, 439.

PLATE XII

EXPLANATION OF PLATE XII

Fig. 1. Stone drainage in a small ravine, showing sloping surface.

Fig. 2. Stone drainage, showing terraced surface.

Fig. 3. Stone packing in old concrete drain leading from terraced stone drain in the background; to save renewal of eroded concrete drain.

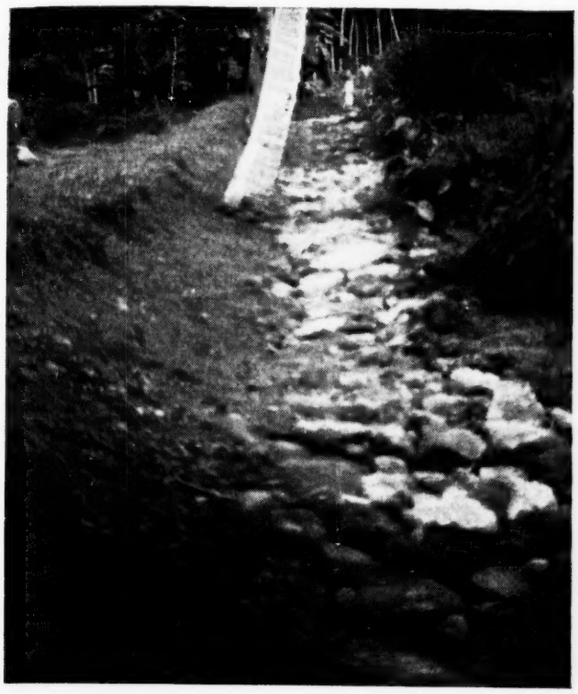


FIG. 1

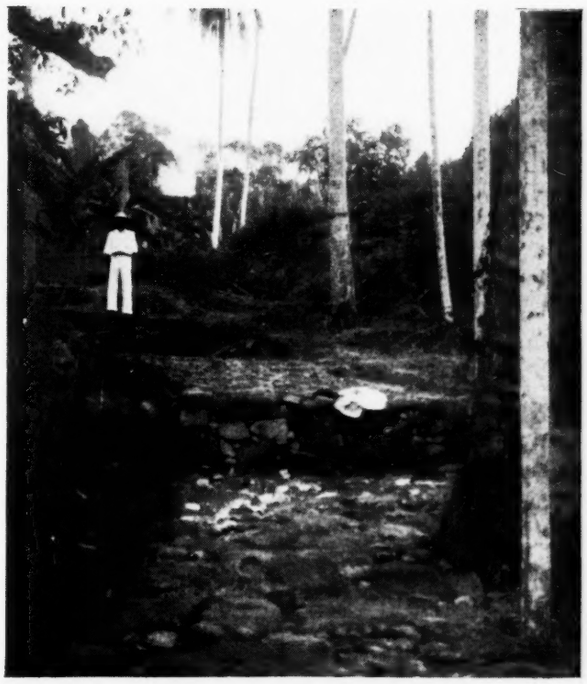


FIG. 2



FIG. 3

H. R. Grubb, Ltd., Poplar Walk, Croydon.

EXPLANATION OF PLATE XIII

Fig. 1. Covered percolation pit with silt-trap on inlet channel.

Fig. 2. Stream-bed, dry as the result of use of percolation-pit method.

Fig. 3. Fascine drains, sticks and lallang grass; 8 months old, opened up for demonstration.

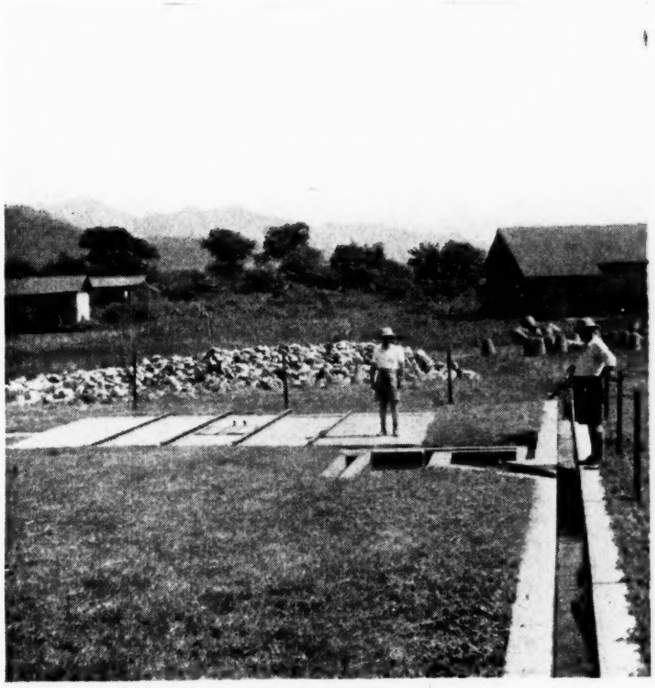


FIG. 1



FIG. 2



FIG. 3

H. R. Grubb, Ltd, Poplar Walk, Croydon.

THE *FUNESTUS* SERIES OF *ANOPHELES* AT KISUMU AND A COASTAL LOCALITY IN KENYA

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INTRODUCTION AND ACKNOWLEDGEMENTS

The present paper records the results of observations made during the visit of one of us (A.M.E.) to Kisumu in May and June, 1936. This visit was made possible by a grant kindly made by the Leverhulme Research Committee, and by the courtesy of Professor W. S. Patton and the Director and Council of the Liverpool School of Tropical Medicine, who granted leave of absence. It was also greatly facilitated by Dr. A. R. Paterson, Director of Medical and Sanitary Services, Kenya, and by the Medical Entomologist, Mr. C. B. Symes. Our best thanks are also due to Captain R. P. Cormack, M.O.H., Kisumu, in whose laboratory investigations were conducted, and to Mr. J. O. Harper, of the Entomological Department, for active co-operation in the work. Dr. E. W. C. Jobson also gave one of us invaluable help when collecting material at 'Msambweni.

Working at Kisumu, one of us (P.C.C.G., 1929) was the first to record a striking larval variation in a *funestus*-like *Anopheles*, and later Leeson (1935) described as *A. funestus* var. *rivulorum* a form which he collected and bred out in Southern Rhodesia. The larva of the Kisumu form was seen to resemble Leeson's variety in the character of its main tergal plates. Material of all the stages was obtained in 1936 for comparison with Leeson's variety. One or two characters not specifically mentioned in the description of that form were noted by one of us from Southern Rhodesian material, for the loan of which thanks are due to Professor P. A. Buxton and Mr. H. S. Leeson.

Later, one of us collected and bred out material of a very similar form from 'Msambweni, in the Digo district, on the coast, about 30 miles south of Mombasa.

The Kisumu material was found to differ in certain particulars from Southern Rhodesian and Digo *rivulorum*, notably in the pupal stage, but most of the differences related to characters that were variable in at least one of the forms. Further, in the Digo material the pupae showed very little divergence from the typical condition, but the wings of the adults exhibited variations not found in either of the other forms. It thus appears that the *rivulorum*-like forms in different localities exhibit certain peculiarities which are not sufficiently marked or constant to indicate more than the existence of local forms of the species. The form occurring at Kisumu showed one striking biological peculiarity—the larvae were always found in association with *Pistia*.

It is now obvious that var. *rivulorum* is quite as distinct from *A. funestus* type form as are many of the oriental species from each other, and we therefore propose to raise it to specific rank.

A large amount of material of the type form of *funestus* was collected, and the preliminary observations recorded by Evans (1936) on variation in the colouration of the tergal plates were followed up.

Anopheles funestus Giles type form

This was found to be by far the commonest form of the series at Kisumu. Amongst large numbers of larval collections made during the past two or three years by members of the Medical Department, all but one or two specimens were the type form. During the present investigation this form only was encountered, except among *Pistia*, and even here the type form occurred in numbers in the shadier situations.

It was obvious that this was the only anopheline of the series which habitually entered huts or tents at Kisumu; the details of the observations leading to this conclusion are given below in dealing with the adult habits of *A. rivulorum*. From this it appears probable that, as in the Transvaal (De Meillon, 1936), *A. funestus* type form is the only member of the series which is practically concerned in the transmission of malaria.

Pale-Plated Larvae. One of us (A.M.E., 1936) has referred to the occurrence among Kenya *funestus* of larvae in which the main tergal plates are largely very pale, with a contrasting dark area proximally. During the present investigations at Kisumu in May and June, it was found that the great majority of the *funestus* larvae collected were of this pale-plated type. It was thought by one of us (P.C.C.G.) that the occurrence of these larvae might be connected in some way with certain anomalies which he had sometimes observed between the curves of malaria incidence and *funestus* density at Kisumu. Observations were therefore made to see whether the pale-plated larvae were associated with any special types of breeding place. It should be mentioned that all conditions intermediate between the extremely pale and dark type of plate are found in nature.

The pale-plated character was apparently not the direct result of any factor

in the breeding places, as larvae with pale plates were found on a few occasions in exactly the same spots as those with dark plates.

In order to test whether or not the character was simply one of individual variation, batches of larvae were reared from eggs, and the characters of the plates in the well-developed fourth stage larvae were observed. All of the egg batches which gave an appreciable number of full-grown larvae turned out to be the pale-plated form. In all cases this character was constant, no dark plates occurring among the batches. The only dark-plated larvae which developed were the two survivors of a batch of which most failed to reach the fourth stage. The numbers here are too small to be of much significance, but the occurrence of the dark-plated larvae shows that the pale condition was not brought about by the artificial conditions under which the larvae were reared. It was found that the distribution of light and dark areas on the plates was remarkably constant throughout any given batch of larvae. This was even the case with two batches of larvae each divided into two lots, the lots being subjected to widely different conditions of illumination during a large part of the larval life.

One of us (P.C.C.G.) has now discovered that the colouration of the plates evidently shows a seasonal change. In one locality near Kisumu it was found that in May almost all the *funestus* larvae were of the extremely pale-plated type, but that larvae collected in the same breeding place on September 9th were mainly of the intermediate or dark types. This question is being investigated further.

Anopheles rivulorum Leeson (Kisumu form)

A considerable number of larvae were obtained for study, and 14 isolations were made.

ADULT. ♀. *Palps.* As in *A. funestus* type form, the actual and relative width of the distal pale bands showed much variation. *Wings* differing slightly from Southern Rhodesian *rivulorum*, as so far known, in having small to minute, pale, basal costal interruptions sometimes present. Third vein much more variable, either all dark or with a pale spot or area which may be half the length of the vein; in all cases where present the pale area was distal in position, as in the type form of *funestus* (Evans and Leeson, 1935).

Pharynx. Number of rods and cones usually about 9–10, and thus less than in the type form of *funestus* (about 11–14). It had been noticed by one of us that in the pharynx of typical *rivulorum* (preparations kindly lent by Mr. Leeson) only 10 well-developed cones were present. Hair-like processes of post-armature ridges as in typical *rivulorum*. These characters can usually be determined satisfactorily in well-macerated dissections of the pharynx mounted in De Faure's fluid, without staining.

ADULT. ♂. *Terminalia.* Agreeing in important characters with that of typical *rivulorum*. Club abnormally slender in some of the specimens.

PUPA. Differing rather markedly from Southern Rhodesian and Digo material as follows :—

Accessory paddle hair simple or bifid (3 or more branches in South African, 2–4 in Digo pupae).

Spine A on V—VII usually relatively shorter than in South African pupae and resembling that of *A. funestus* type form ; that on VII about 1/3–2/5 the length of the segment in 11 out of 12 pelts in which clearly displayed. In a single pelt it approached half the length of the segment.

LARVA. *Colour.* During life the larvae almost always show a markedly banded appearance, the banding being produced by whitish pigment mainly on the dorsal surface of the thorax and abdominal segments II and V. It was also found that *A. funestus* type form commonly showed similar pigmentation, but the abdominal bands might be on either segments II and V or II and VI. *Shape.* Full-grown larvae differed in shape from those of *A. funestus*, the thorax being much less broad ; this character was first noticed by Mr. J. O. Harper, who was able to distinguish the larvae at sight by this character, his determinations being confirmed by microscopical examination.

Head. Fronto-clypeal markings. Anterior half of fronto-clypeus in 90 per cent. of larvae either almost entirely dark or with a large dark area, much deeper than the dark band in *A. funestus* type form, which Southern Rhodesian larvae resemble in this respect.

Thorax. Bases of inner and middle shoulder hairs almost always separate, sometimes widely so, and base of inner commonly much smaller than in *A. funestus* type form. The bases were joined in only 4 out of 48 cases in mounts in which they were well displayed ; both pairs were joined in one specimen only. This character was pointed out by one of us (P.C.C.G., 1929) in describing his a-typical *funestus*.

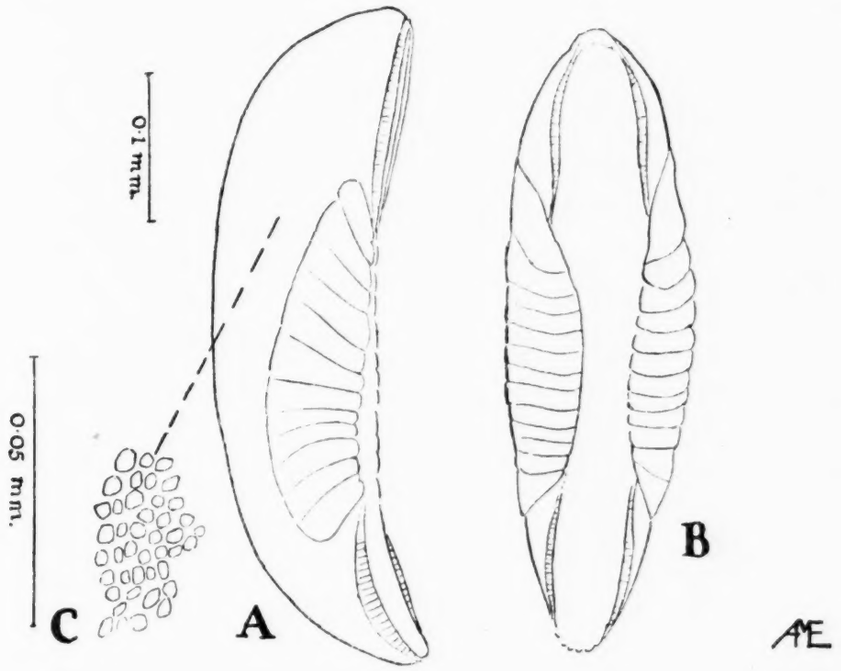
Abdomen. Paired accessory tergal plates normally absent ; in one example, out of 20 pelts and larvae in which the character could be very clearly seen, small paired plates were present on IV—VII. In Leeson's Rhodesian material paired plates were always present, at least on some of the segments.

EGG (text-fig.). The following description is based on a number of well-preserved eggs obtained by one of us (P.C.C.G.) from a ♀ caught out-of-doors at Kajulu. The ♀ was determined on external and pharyngeal characters.

The egg is of the same type as that of *A. funestus* type form, but differs markedly, as follows. *Frills* better developed, especially the posterior, and enclosed areas much broader. Bosses of convex surface much smaller than those of the type form (Gibbins, 1933 ; Evans and Leeson, 1935), but about equal to those of *A. funestus* var. *confusus* (Evans and Leeson) and irregular in size and arrangement. Floats with about 12–13 float ridges. The eggs are somewhat shorter than the average *funestus* eggs, the greatest length measured being 0·45 ; this character may, however, be variable.

HABITS OF ADULTS. These were studied in the vicinity of a large swamp

at Kajulu, in which *A. funestus* type form and *rivulorum* were breeding in large numbers. One of us (P.C.C.G.) had a tent erected on the banks of the swamp, and two boys were detailed to catch all adult *Anopheles* that entered during a whole night. Adult catches were also made on two occasions in a number of native huts near this swamp. The *funestus*-like ♀♀ were determined by pharyngeal characters or by rearing batches of larvae; the egg characters were also examined in many cases. Altogether 5 ♀♀ caught in the tent and 36 from the huts were identified, and in all cases were found to be the type form of *funestus*. It is clear from these results that the Kisumu form of *rivulorum* is not an habitual



TEXT-FIG. *Anopheles rivulorum* Leeson (Kisumu form). A.—Egg, mainly lateral aspect B.—Egg, upper aspect; C.—Bosses on convex surface, more highly magnified.

house-frequenter. During July, 1936, one of us (P.C.C.G.) instituted the catching of *funestus*-like adults in outdoor haunts near the swamp at Kajulu. Altogether 7 ♂♂ and 5 ♀♀ were determined by morphological characters; most of them were collected in a deep hollow near the ground in the trunk of a large tree close by the swamp. All but 1 ♂ and 1 ♀ were found to be *rivulorum*.

RELATION TO MALARIA. From the fact that the ♀♀ do not habitually enter huts, and were not even attracted by Africans in a tent close to a prolific breeding place, it is more than probable that this anopheline does not play an important part in the transmission of malaria at Kisumu.

BREEDING PLACES. In the three localities where larvae of this form were taken in 1936, they were found in beds of the water-lettuce, *Pistia stratiotes*. The presence of this plant in the breeding places of a-typical *funestus* was

noted formerly by one of us (P.C.C.G.), and it was by following up this clue that we were able recently to track down the larvae. The three types of breeding place found were as follows : (1) A bed of *Pistia* in a native fish-trap in the outer fringe of the *Papyrus* belt of the lake shore (Kavirondo Gulf, Lake Victoria) (Pl. XIV, figs. 2 and 3). This water is subject to some disturbance from wave and wind action. The salinity of the lake-water was found to be 1·5 parts of chlorine per 100,000 water. (2) In beds of *Pistia*, in a permanent swamp, 7 miles from the lake at Kajulu (Pl. XIV, fig. 1). The water here was stagnant, foul-smelling and of relatively high salinity ; during the dry spell, 27·5 parts chlorine per 100,000 water, and after rain, 10 parts chlorine. (3) In *Pistia* in a slowly running river about 10 miles from Kisumu, partly shaded by tall reeds.

In all these sites the *rivulorum* was accompanied by the type-form of *funestus*. The former, however, occurred in the open, central parts of the *Pistia* beds, while the latter was found at and near the shaded margins, the distribution of the two forms overlapping a short distance from the banks.

Anopheles rivulorum Leeson (Digo form)

Larvae of this form had been collected previously in the Digo district (about 30 miles south of Mombasa) by one of us (P.C.C.G.) and by other members of the Kenya Medical Department. Material from this district collected and bred out by one of us (A.M.E.) in August, 1936, showed a few differences from Southern Rhodesian *rivulorum*, but in most respects agreed more closely with this, the typical form, than with Kisumu specimens. The following are the main points of difference from these two forms of *rivulorum*.

Adult. *Wings*. With well-marked basal costal interruption commonly present. Third vein extremely variable, showing all the conditions found in Kisumu *rivulorum*, but in two adults differing markedly from *funestus*, and resembling the more characteristic specimens of *A. leesoni* in being pale with a small equal dark spot towards each end. In another example a small pale area was median in position, thus resembling *A. leesoni* and differing from *funestus* and the known varieties.

Pupa. Practically as in typical *rivulorum*, accessory paddle hair split into 2–4 branches.

Larva. The larvae resembled the Kisumu form in general appearance.

Head. Fronto-clypeus, as in Kisumu form, usually with a broad dark area on anterior half.

Thorax. Bases of inner and middle shoulder hairs separate in all cases where clearly seen.

Abdomen. Paired accessory tergal plates were present or absent in about equal numbers of larvae bred out, the form being almost intermediate in this respect between those from Southern Rhodesia and Kisumu.

Breeding Places. At 'Msambweni, Digo district, August, 1936, larvae

were found in two swamps without *Pistia*. In both swamps larvae of the type form of *funestus* were found, but in other swamps inhabited by the latter *A. rivulorum* did not occur. It had been noted previously by one of us (P.C.C.G.) that in this district larvae of the latter were found in the absence of *Pistia*. There would thus appear to be a biological difference between the Digo and Kisumu forms.

Habits of Adults. Dr. Jobson kindly arranged for the collection of adult anophelines from a house close by a swamp at 'Msambweni, where some larvae of *A. rivulorum* were taken together with numerous *funestus* larvae. The pharynxes of 16 ♀♀ were examined by one of us (A.M.E.), and 15 of these were definitely the type form of *funestus*. The remaining specimen was almost certainly the type form. These observations are not conclusive, but tend to show that *A. rivulorum* is not a habitual house-frequenter in this region.

'Inimical' Aquatic Plants. It is interesting to record that at 'Msambweni numerous larvae of *A. funestus* were found in a swamp among a dense growth of *Chara* sp. In another swamp, larvae of this form and of *A. rivulorum* were found among bladderwort, *Utricularia* sp.—almost certainly *U. stellaris* (kindly determined by Professor J. MacLean Thompson). Some of this plant from the breeding place was examined, but no larvae were seen in the bladders. On the other hand, a piece of the plant placed overnight in a small vessel together with living *Anopheles* larvae caught a number of moderate-sized specimens. The following morning these were seen in the bladders, some with their heads projecting from the openings. Were it not for the negative field observations, it might have been concluded that the *Utricularia* would be a powerful agent in controlling the larvae of *Anopheles*.

SUMMARY AND CONCLUSIONS

The anopheline described by Garnham (1929) as a-typical *funestus* agrees in essential characters with *A. rivulorum* Leeson (*A. funestus* var. *rivulorum*) from Southern Rhodesia. The Kisumu form exhibits a marked peculiarity in its choice of breeding places, being closely associated with *Pistia stratiotes*; it appears, therefore, to represent a distinct biological form of *rivulorum*, and is also distinguished by certain minor differences, especially in the pupal stage. As these differences concern characters that vary in one or more forms of *rivulorum*, they are not considered to be of varietal value. Eggs of Kisumu *rivulorum* were obtained and are described.

The Kisumu form of *rivulorum* was found not to be a house-frequenter, and is therefore in all probability not seriously concerned in the transmission of malaria.

In the Digo district on the coast, a form of *rivulorum* was studied which resembled Southern Rhodesian material more closely in the early stages than does the Kisumu form. A striking variation was the occurrence of specimens

(reared from larvae) in which the third vein resembled that of characteristic light-veined examples of *A. leesoni*.

A. funestus type form was found to be by far the commonest member of the series in both localities and, in Kisumu at least, was the only one found in human habitations.

The possible significance of very pale tergal plates in larvae of *Anopheles funestus* was partially investigated.

Breeding of the *funestus* series in close association with the aquatic plants *Chara* and *Utricularia* is recorded. The latter plant was found to capture several larvae under artificial conditions.

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EXPLANATION OF PLATE XIV

Fig. 1. Breeding place of *A. rivulorum* Leeson (Kisumu form) in *Pistia*-covered swamp at Kajulu. Larvae of *A. funestus* Giles type form were found near the margins of the swamp.

Fig. 2. *Pistia* bed in outer fringe of *Papyrus* at edge of Lake Victoria. Larvae of *A. rivulorum* were found in the most open part of this inlet.

Fig. 3. *Pistia* near edge of inlet (fig. 2). Larvae of *A. funestus* type form were found here, close to the banks.



FIG. 1



FIG. 2



FIG. 3

H. R. Grubb, Ltd., Poplar Walk, Croydon

TRYPANOSOMIASIS OF STOCK IN MAURITIUS*

III.—THE DIAGNOSIS AND COURSE OF UNTREATED *T. VIVAX* INFECTIONS IN DOMESTIC ANIMALS

BY

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Sundry animals were infected by syringe inoculation with *T. vivax* strains maintained at the laboratory, and the course of the untreated disease was studied in them over lengthy periods. These animals were kept at the laboratory in efficiently fly-proofed stables, and were housed and cared for under conditions, it is safe to say, obtaining nowhere else in the island. While race-horses, which are imported into Mauritius annually in small numbers, are stabled and maintained with the utmost concern for their welfare, this can by no means be said to be the case in general with cattle, sheep, goats and pigs. It is obviously economically impossible to devote that care and attention to their welfare which are devoted to the few occupants of the racing-stables. The experimental animals were procured from various parts of the island, and were segregated and examined daily over a period for possible existing trypanosomiasis. In no instance was evidence of previous infection obtained, although it is impossible absolutely to exclude a previous *T. vivax* infection, more particularly in the case of the cattle ; but this was, as far as possible, prevented by securing the animals from estates where there was no history or evidence of the recent presence of this trypanosome, and by selecting only young animals which presumably had had a minimum opportunity of acquiring a natural infestation. The course of the subsequent experimental infections observed at the laboratory leads to the belief that neither *T. evansi* nor *T. vivax* had previously affected any beast employed in the experiments.

STRAINS OF *T. VIVAX* EMPLOYED

The Highlands strain. *T. vivax* was originally obtained from an ox found naturally infected at Highlands Sugar Estate, which is situated in about the centre of the island. Blood from the animal was inoculated into a young goat by the intraperitoneal route on July 9th, 1934, and subsequently the strain was passed by subcutaneous inoculation through an ox, another goat, a sheep and a second ox. Several varieties of animals were inoculated from each of these passage animals, and the results of attempted infection of the smaller laboratory animals have been recorded in a previous communication (Adams, 1935*a*) ; it suffices here to reaffirm that small laboratory animals of all kinds were found refractory to true infection with the strain.

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* From the Bacteriological Laboratory, Medical and Health Department, Mauritius.

Six goats were readily infected with small quantities of blood obtained from the oxen. Each of the six died, five of acute trypanosomiasis in periods ranging from two weeks to one month. The sixth goat, in contradistinction to the other five, ceased to show parasites in its blood or in gland puncture material about four months after the date of infection ; and none were seen for another four months. The animal then suddenly developed what appeared to be an acute bilateral parotitis and became acutely ill ; the following day numerous parasites were readily found ; a similar state of affairs obtained the next day ; and the goat died on the ensuing day, with great numbers of trypanosomes in all its tissues, but after apparent recovery from the parotid trouble, as judged by the almost entire subsidence of the swelling.

Two sheep were infected subcutaneously with a relatively small number of trypanosomes from an ox. One animal died in about a month of acute trypanosomiasis, and the other in two months ; in each case the blood at death contained numerous trypanosomes.

Three oxen were infected with this same strain by subcutaneous injection of small quantities of infected blood from goats or sheep. The first of the three was found to have trypanosomes irregularly in its peripheral blood and in gland puncture material until two months before its death, which resulted from an accident nine months after infection. The animal at this time was free from all symptoms, and apparently in good health and condition. It grew and put on weight steadily throughout the period of observation, except during the first three weeks of the infection when it had some fever and diarrhoea and lost condition. A second ox for two months after infection showed parasites irregularly ; it then became entirely negative, as far as regular examination could show, for the next six months. After this period a considerable number of parasites of the same strain obtained from a sheep was injected subcutaneously without apparent change or ill-effect. For another three months all examinations consistently failed to reveal the presence of trypanosomes, until finally the animal fell and damaged a limb. The following day large numbers of *T. vivax* were found ; some fewer were seen the next day, when the animal was destroyed in view of its injury and possible suffering. The third ox after showing parasites for six weeks, irregularly, became negative to all ordinary examinations, and has remained so, in seemingly excellent health, to the present time, some sixteen months later.

Two pigs failed to become infected after the injection of a considerable amount of infective inoculum, daily blood examination for four months failing to yield any trypanosomes on long search.

The Mon Desert strain. An ox naturally infected with *T. vivax* was found on an estate in the south end of the island, some fifteen miles distant from Highlands. Blood was taken from a jugular vein and inoculated into a goat, and the strain was then passed through another ox, into sheep, goats and pigs. Another ox was infected from one of the sheep.

Three goats were infected from oxen with this strain, and, of the three, two died of trypanosomiasis—one within two weeks, and the other six weeks from the date of infection. The third animal lived for some seven months, during the first four of which trypanosomes could be recovered irregularly. For the next three months none were seen ; and finally the animal suddenly sickened and died for no very obvious reason and without the reappearance of parasites.

Two sheep, also infected from the oxen, died of acute trypanosomiasis, the first in two weeks, and the second two months after infection. A couple of pigs were inoculated with massive doses of parasites, but failed to develop any infection.

The three oxen infected with this strain are all alive and well, 16, 18 and 20 months respectively after being infected. In each case characteristic *T. vivax* were found for some weeks after inoculation ; they then irregularly and gradually disappeared, and have not recurred. One of the animals when apparently fully recovered was inoculated with many parasites of the Highlands strain ; but this had no visible effect, and no parasites were seen in the animal subsequently.

In every case where an experimental animal, be it sheep, goat or ox, survived for a period longer than three or four weeks, the trypanosomes disappeared, sometimes for days or weeks at a time, from the peripheral circulation and from gland puncture material. The numbers of trypanosomes present, on those days when they could be found by direct microscopical examination, varied very greatly from day to day ; no definite cycle in relation to their numerical intensity could be determined. In general, parasites completely vanished from the blood and glands of the cattle within a couple of months, and did not return, as far as our examination technique could show, under normal laboratory conditions.

GLAND PUNCTURE AND BLOOD EXAMINATION IN DIAGNOSIS

Gland puncture and the examination of the resultant material have been extensively practised in the diagnosis of human trypanosomiasis for many years ; the technique is simple, and is freely recognized as being of very considerable value in the diagnosis of sleeping sickness in man. The method does not appear to have received much attention in the literature on the trypanosomiases of stock until comparatively recent years. Hornby (1921), writing of *T. vivax* in stock, records that he has found the examination of the gland juices more likely to yield positive results than that of the blood in cattle ; he recommends both examinations as a routine procedure in searching for this condition. Du Toit (1929) considers gland puncture a valuable method of detecting *T. vivax* infestation in bovines, and states that ' the chances of diagnosing an infection in the gland smear as compared with the blood smear are as 12 to 1.' Parkin (1931) undertook a study of the relative frequency of trypanosomes in blood and in

gland smears in *T. vivax* and in *T. congolense* infections in cattle, and concluded that in the case of experimental *T. vivax* infections the parasites appeared in the glands about one week earlier than in the peripheral blood ; he affirms that the diagnosis of *T. vivax* infestation should be centred on gland material examination, blood smear study ' being of practically no assistance.' Van Saceghem (1934) mentions the well-known difficulty in detecting *T. vivax* infections in bovines by blood examination, either in fresh or in stained preparations, owing to the transitory appearances of the parasites in this medium. He states that when the trypanosomes cannot be found in the peripheral blood they may be recovered almost invariably in the lymph glands. He regards gland puncture as the correct standard method of arriving at a diagnosis of *T. vivax* in bovines, with blood examination in a very subsidiary rôle.

There appears to be considerable unanimity of opinion among these workers that *T. vivax* is invariably considerably more readily found in the gland juices than in the peripheral blood in cattle ; and it is, moreover, implied by several of them that gland puncture is an efficient method of detecting this trypanosome. Van Saceghem goes so far as to say ' qu'à de très rares exceptions près, le trypanosome se trouve toujours dans le suc ganglionnaire.' These conclusions have been found somewhat at variance with those drawn during the syringe transmissions of local strains of *T. vivax* in Mauritius, and it has been deemed advisable to pursue the matter further in view of its manifest importance.

In a number of experimental infections in cattle, goats and sheep, both the peripheral blood and material obtained by puncture of the glands were examined at intervals throughout the whole courses of the infections. Blood examination was performed daily by snipping an ear with scissors and making wet cover-slip preparations of the blood. Gland juice was collected by puncturing one of the prescapular glands with a stout needle on a syringe, and by blowing the material obtained on to a slide and examining in a similar manner. For the first few days the glands were examined daily, taking each side on alternate days ; subsequently they were punctured usually a couple of times a week only, again on alternate sides, as it was felt that too frequent puncture caused unnecessary pain and gave rise to the possibility of secondary infection. The prescapular glands in each species of animal successfully infected with *T. vivax* enlarged within a week of the infective inoculation ; in the case of cattle, these glands attained the size of a tangerine orange with considerable rapidity ; they remained enlarged and fairly soft for several weeks, and then diminished in size as the animal began consistently to fail to yield parasites. There does not appear to be any necessity to trace in great detail all the observations made on each animal over a period of many months ; these have already been summarized elsewhere (Adams, 1936). A few selected cases may be considered as being more especially relevant to the present enquiry.

An ox (ox A) was inoculated with the Highlands strain of *T. vivax* from a goat, and affords information of profound interest. The animal was infected

on October 17th, 1934 ; its blood contained trypanosomes on the seventh day after inoculation, and remained positive continuously until November 17th, i.e., one month later ; it then was somewhat irregularly positive until December 22nd, 1934, when it became negative and consistently remained so. Regular gland puncture of this particular beast was not started until January 11th, 1935, by which time the blood had been negative for $3\frac{1}{2}$ weeks ; but it was performed thereafter every 3–5 days in conjunction with daily blood examination. Blood, containing parasites of the homologous strain from a sheep, was inoculated on May 9th, 1935, without causing recurrence of parasites in the blood or gland juice. On August 8th, 1935, or after the animal had failed to yield any parasites whatsoever in the blood or glands for eight months, this ox severely damaged a leg by falling on a slippery floor. On the following two days trypanosomes were once more readily found in both the peripheral blood and in the gland juices, and on the next day the animal had to be destroyed owing to the nature of the injury. From observation on this beast, it is apparent that repeated gland puncture proved as valueless as blood examination in the diagnosis of a latent *T. vivax* parasitization which undoubtedly was present throughout.

A goat (goat I) afforded information of a similar nature. This animal was inoculated with the same strain as that used for the ox mentioned above. After four months it appeared to throw off the infection entirely, as judged by its physical condition and the complete failure to find parasites in the blood or gland juices examined at frequent intervals. What appeared to be an acute parotitis developed after a further four months of good health and apparent freedom from parasites ; the organisms immediately reappeared in both the peripheral blood and gland puncture material, and were found there in large numbers for a couple of days before the animal died. This once again demonstrates the failure of gland puncture as a reliable technique in the diagnosis of a cryptic infection with *T. vivax*, under the conditions of experiment.

Turning now to the consistency of the results obtained with gland puncture in an active established infection: certain of the workers state that when the blood is positive the glands are also positive and show greater concentration of the parasites ; they furthermore imply that when the blood temporarily becomes negative the glands still yield trypanosomes. After the inoculation of *T. vivax* into sheep, goats and cattle at this laboratory it has been observed that trypanosomes often appear some 24 or 48 hours in the glands before they appear in the peripheral blood ; but this is by no means an invariable rule, parasites frequently appearing in each for the first time on the same day. In no instances so far have the glands failed to yield trypanosomes on the day the blood has first been found to contain them. Later in the infections it has been found that, as a general rule, the presence of parasites in the blood has been associated with their presence in the glands. Some exceptions also occurred to this rule, and it is interesting to note that six of these were in sheep, apparently the most susceptible animals employed in the work ; two in goats, the next most susceptible ; and only one

in cattle, the most resistant, pathogenically speaking. The results were checked by repetition of the punctures to avoid the possibility of experimental error, but it was determined that although on these particular occasions the blood contained relatively numerous trypanosomes none could be found in the glands. It was frequently observed that when trypanosomes cannot be found in the peripheral blood they may equally be absent from gland puncture material, even during the first few weeks of the infections ; but nevertheless the glands were on the whole more frequently positive than the blood during this period. Both became negative for days, weeks or even months at a time in the later stages of the condition.

Parkin (1931) publishes some figures recording his findings in respect of the numbers of trypanosomes in experimental *T. vivax* and *T. congolense* infections in cattle. Those given in respect of the numbers of parasites found in the blood and the glands in *T. vivax* infections support his own statement that gland smears contain very considerably greater numbers of trypanosomes than does the peripheral blood on microscopical examination ; in actual fact he found roughly fifty times the concentration in a given volume of the former material than he did in the latter. In Table I are recorded the findings at this laboratory in a sheep infected with the Highlands strain of Mauritian *T. vivax* ; these are typical of those obtained with three other sheep.

From the study of this Table it will be seen that in this sheep the numerical ratio of parasites found in the blood preparations to those found in the gland juice preparations varies very considerably throughout the course of the infection. On the whole, the concentration of trypanosomes is usually greater in the blood than that in the glands ; on May 23rd, 1935, and May 30th, 1935, none were recovered from the glands, although the blood on these dates contained considerable numbers. A reverse condition is seen on June 14th, 1935, when one parasite a field was seen in gland puncture material and none in the peripheral blood ; this was one of the two occasions in this particular animal on which the blood was negative while a gland was found positive. On two further occasions the glands yielded no parasites, but the blood also was negative, although in each case the latter contained parasites very shortly afterwards. The results obtained were so consistent throughout the observations on each of four sheep that it was evident that gland puncture would not in practice prove superior to blood examination in making a diagnosis in this particular variety of animal.

In Table II are set forth in a similar manner the findings in the case of a goat infected with the same strain of *T. vivax*. It will be observed that trypanosomes appeared for the first time in the gland material two days before they were seen in the blood. After this, both tissues were positive each day when both were examined, with one exception, when no parasites were recovered from either. The numerical concentration was usually greater in the gland material than in the blood, but either blood or gland examination afforded a positive diagnosis without undue difficulty on most days.

TABLE I

To show the results* obtained by blood examination and by gland puncture throughout the course of an untreated *T. vivax* infection in sheep I (Highlands strain)

Date	Fresh blood preparations	Fresh gland preparations
18.4.35	1 c.cm. blood of ox D (1/2) inoculated subcutaneously	
19, 20, 21.4.35	neg.	neg.
22.4.35	neg.	1/20
23.4.35	1/10	1/10
24, 25.4.35	1/5 ; neg.	
26.4.35	neg.	neg.
29, 30.4.35	1/100 ; 2/1	
1.5.35	20/1	1/100
2.5.35	30/1	
3.5.35	30/1	1/5
4, 5.5.35	2/1 ; 1/1	
8.5.35	5/1	1/10
9.5.35	5/1	
10.5.35	1/100	1/5
11, 12, 15.5.35	neg.	
16.5.35	1/5	1/2
17, 18, 20.5.35	5/1 ; 2/1 ; 1/50	
21.5.35	neg.	neg.
22.5.35	1/20	
23.5.35	1/20	neg.
25.5.35	1/2	
27.5.35	1/5	1/1
28.5.35	1/10	
30.5.35	10/1	neg.
4.6.35	1/3	1/100
5, 6.6.35	1/1 ; 1/50	
7.6.35	30/1	1/20
8.6.35	1/3	
10.6.35	1/50	1/20
12.6.35	20/1	
14.6.35	neg.	1/1
15, 17.6.35	neg. ; 1/10	
18.6.35	1/10	1/2
19.6.35	2/1	
20.6.35 Animal died		

* 1/50 means one parasite in 50 1/6″ field with a 4 Zeiss ocular ; similarly the other figures refer to trypanosomes per field ; ' neg. ' means no parasites found after a thorough search of at least one whole 7/8″ cover-slip preparation.

TABLE II

To show the results obtained by blood examination and by gland puncture throughout the course of an untreated *T. vivax* infection in goat 5 (Highlands strain)

Date	Fresh blood preparations	Fresh gland preparations
1.3.35	0·3 c.cm. blood of ox D (1/20) subcutaneously	
2, 3, 4, 5.3.35	neg.	neg.
6.3.35	neg.	1/200
7.3.35	neg.	neg.
8.3.35	1/100	1/100
9, 11, 12, 12.3.35	1/10 ; 1/5 ; 30/1 ; neg.	
14.3.35	neg.	neg.
15, 16, 18, 19.3.35	1/20 ; 20/1 ; 1/100 ; 1/20	
20.3.35	1/50	1/4
21.3.35	1/50	
22.3.35	1/100	1/5
23, 25.3.35	1/1 ; 20/1	
26.3.35	1/1	5/1
27, 28, 29.3.35	1/20 ; 20/1 ; 30/1	
30.3.35	30/1	30/1
1.4.35	Animal died with many trypanosomes in all tissues	

Observations made on an ox are recorded in Table III. This animal was infected with the Mon Desert strain of *T. vivax* from a goat to which it had proved rapidly fatal. Trypanosomes appeared in both the blood and the gland juices of the ox for the first time on the same day, i.e., on the fourth day from infection. Subsequently, when parasites were found in the peripheral blood they were also seen in gland puncture material, sometimes in slightly greater numbers. On three occasions only were parasites readily recovered from the glands when they could not be found in the blood preparations (March 29th, 1935, April 2nd, 1935, and April 6th, 1935). The glands of this particular ox were punctured in all some sixty odd times ; after April 11th, 1935, they, together with the blood, were consistently negative to microscopical examination, except on three days some three weeks later (April 30th, 1935—May 2nd, 1935), when both contained parasites. From the figures it cannot be said that gland puncture is greatly superior to blood examination in the recovery of the organisms from this animal ; the numerical intensity of the parasites recovered by each means closely approximates, and neither technique affords any indication that an infection exists during the three-week-period before the parasites reappeared for the last time. Gland puncture involves more time and trouble than does the rapid cutting of an ear and the moistening of a slide with blood ; assistance is necessary to hold the beast, and further assistance to attend to the slides and look after the paraphernalia required in the preparation of the syringe and needles

TABLE III

To show the results obtained by blood examination and by gland puncture throughout the course of an untreated *T. vivax* infection in ox E (Mon Desert strain)

Date	Fresh blood preparations	Gland preparations
4.3.35	4 c.cm. blood of goat 4 (1/20) subcutaneously	
5, 6, 7.3.35	neg.	neg.
8.3.35	1 seen	1 seen
9, 11, 12, 13.3.35	neg. ; 1/10 ; 20/1 ; neg.	
14.3.35	1/100	1/5
15, 16, 18, 19.3.35	1/20 ; 5/1 ; 3/1 ; 5/1	
20.3.35	25/1	40/1
21.3.35	20/1	
22.3.35	1/2	3/1
23, 25.3.35	5/1 ; 20/1	
26.3.35	25/1	20/1
27.3.35	1/20	
28, 29.3.35	neg. ; neg.	1 seen ; 1/2
30.3.35	neg.	
1, 2.4.35	1/5 ; neg.	1/5 ; 1/10
3, 4.4.35	1/100 ; neg.	
6.4.35	neg.	1/100
8, 9.4.35	1/2 ; 1/100	
10.4.35	1/2	1/2
11.4.35	20/1	
12.4.35	neg.	neg.
13, 14.4.35	neg. ; neg.	
16.4.35	neg.	neg.
17.4.35	neg.	
18.4.35	neg.	neg.
20.4.35	neg.	
23.4.35	neg.	neg.
24, 25.4.35	neg. ; neg.	
26.4.35	neg.	neg.
27, 28, 29.4.35	neg.	
30.4.35	1/10	1/10
1, 2.5.35	1/2 ; 1/5	
3.5.35 until 12.8.36	Both blood and gland examinations consistently negative	

when several animals have to be examined. It thus has certain disadvantages in comparison with blood examination in the field, and these disadvantages do not appear to be outweighed by the advantages of the method in ordinary clinical practice.

In view of previous experience with ox A and goat I, already mentioned, I personally have little doubt that the ox mentioned above still harbours *T. vivax*, in spite of all efforts up to a recent date to demonstrate its presence. Unfortunately, I myself no longer have access to the animals, owing to my permanent departure from the colony, but it would be exceedingly interesting to study the effect of making an animal in this state perform hard work in harness. Alternatively, the injection of a drug, like histamine, to produce shock might well be tried in an endeavour to break down the probable balance existing between such oxen and their parasites, and a technique employing this or some similar substance may prove of considerable diagnostic value in revealing latent or cryptic infections with sundry trypanosomes to which animals may develop a state of tolerance-immunity.

SUMMARY

1. Two strains of Mauritian *T. vivax* were maintained in ruminants by subcutaneous inoculation of small quantities of blood from animal to animal.

2. The experimental animals were segregated and kept in a number of efficiently fly-proofed stables under good conditions of maintenance.

3. Sheep were found to be highly susceptible to the infection. Four animals died of acute trypanosomiasis inside a period ranging from two weeks to two months after the infecting inoculation.

4. Goats were also highly susceptible and died in the majority of cases from the disease in a similar period. Some few, however, survived the immediate effects, and appeared to develop a considerable tolerance to the parasite.

5. Cattle were readily infected, but proved relatively resistant to the effects of infection. All developed a high degree of tolerance-immunity after some weeks of subacute febrile disturbance.

6. Pigs proved refractory to infection, as were all the ordinary smaller laboratory animals.

7. Attempted superinfection of two cattle with either a homologous or a heterologous strain of the same parasite failed to produce any apparent result.

8. Gland puncture was explored as a method of diagnosis of the experimental infections in cattle, sheep and goats. It was found that on occasion trypanosomes were recovered 24 or 48 hours earlier in the gland juices than in the peripheral blood of these animals.

9. In sheep and goats which eventually died from acute or subacute trypanosomiasis, gland puncture did not prove superior to blood examination as a diagnostic technique. In several instances it was inferior both in regard

to the frequency of positive results and in the numerical concentration of parasites.

10. In cattle, trypanosomes were frequently present in the prescapular glands in greater concentration than in the peripheral blood during the first few weeks of the subacute stage of the infection. After this, each method gave closely similar findings. In certain cases trypanosomes were found in considerable numbers in both the blood and gland juices, after being absent for very prolonged intervals, as a result of lowering of the general resistance by accidental injuries.

11. Neither gland puncture nor peripheral blood examination is an efficient method of excluding a latent or chronic *T. vivax* infection in ruminants.

12. It is suggested that the administration of some drug, such as histamine, to produce severe systemic shock might profitably be employed to break down temporarily the balance existing between trypanosome and mammalian host and to reveal such latent infections. These cryptic infections have been shown to persist certainly for many months and probably for years, and are of very considerable importance in that they constitute a hidden reservoir of infection in epizootic or enzootic areas.

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TWO NEW ANOPHELINE MOSQUITOES FROM KENYA

BY

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(*From the Department of Entomology, Liverpool School of Tropical Medicine*)

(*Received for publication* 6 *November*, 1936)

The first of the species here described was collected during my recent visit to Kenya.* The larvae were found by Mr. J. O. Harper at Kitale, and were examined by us on the spot with a binocular microscope. They were seen to differ from those of any known African larvae, and a considerable amount of material was taken back to Kisumu and a good series of isolations obtained. The adult proves to be a very distinct member of group *Myzomyia*.

Larvae of the second species have been collected from time to time at Isiolo in Kenya, and several of them sent to me in 1935 by Mr. Symes appeared to represent an undescribed species. In June and July, 1936, some isolations were made by the Department of Medical Entomology. Four bred adults, two of them accompanied by their larval and pupal pelts, were sent to me very recently, and these prove to be of great interest. By the pleural hairs the larvae obviously belong to subgroup II of group *Myzomyia*, and they are exceptional in this group in possessing very wide tergal plates which give them a superficial resemblance to *A. longipalpis*. The adults, on the other hand, are almost indistinguishable externally from *A. funestus* type form, and even the terminalia approach those of *funestus* very closely.

Anopheles (Myzomyia) harperi sp.nov.

A medium-sized and rather a-typical member of group *Myzomyia*, subgroup I. ♀ *palpi* with broad apical pale band, but subapical usually somewhat narrower than intervening dark band. First vein with pale interruption in third main dark area ; third vein pale, with small dark spots near the ends. Mesonotal scales well developed. Hind tarsi without distinct rings on last 3 segments ; rings on first 2 segments sometimes barely distinct. Pharynx with small lateral flanges. Larvae with the feathered pleural bristles with relatively few branches and lateral bristle on abdominal segment III very sparsely feathered.

ADULT. ♀. *Head* (fig. 1). Pale scales mainly white. Frontal tuft with about 8–12 modified white bristles. Antenna with numerous pale scales on first flagellar segment and sometimes a few on second. Palpal index about 0·35–0·43. Palpi with apical band broad, involving the whole of the fifth and apex of fourth segment ; subapical band narrower than apical, usually less than

* See acknowledgements in paper by Evans and Garnham, p. 521 *supra*.

intervening dark band and involving the base of the fourth and apex of third segments ; proximal band very narrow, at apex of second segment.

Pharynx. Lateral flanges very small, about equal to those in *Neomyzomyia*. Only 7–9 pairs of well-developed rods and cones. Some, at least, of cones showing two rows of crest spines less widely separated than in *Neocellia* ; filaments narrow to moderately wide, serrated and sometimes split distally. Rods without well-marked bulbous bases ; filaments about equal to those of cones. Hairs of post-armature ridges mostly short, but one or two may be developed into supernumary filaments.

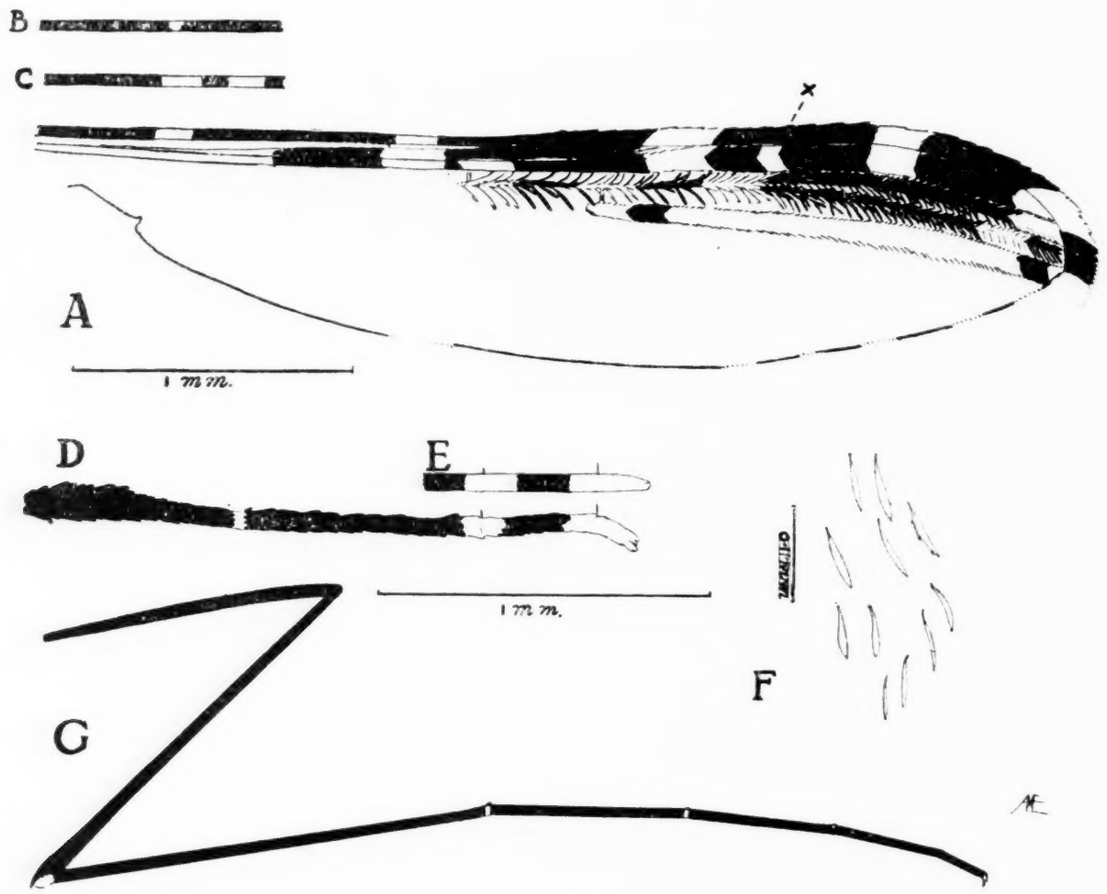


FIG. 1. *Anopheles harperi* sp.nov. A.—Wing of ♀, fourth to sixth veins omitted ; B and C.—Base of costa from other specimens ; D.—♀ palp ; E.—End of palp with unusually broad subapical pale band ; F.—Scales from mesonotum (drawn *in situ*) ; G.—Hind leg. *x*.—Pale spot in third dark area on first vein.

Thorax (fig. 1). Dark lateral areas of mesonotum relatively narrow. Median area mainly clothed with creamy-white scales, some of moderate width, others narrow to very narrow. The broader type of scale predominating on anterior half, but in some specimens largely replaced by very narrow scales posteriorly ; in other specimens the scales are all mainly of the broader type. Fossae and prealar areas without scales. Prosternal hair single and more or less

decumbent. One or two small spiracular hairs. About 3–4 upper mesepimeral hairs.

Abdomen. Dark brown ; scales absent, even on cerci.

Legs (fig. 1). Femora without definite pale markings. Tibiae dark at base, indications of pale apical spots, most distinct on those of fore-legs. Front tarsi with a few pale scales at the apices of the first three segments, usually forming distinct rings on the first two. Mid tarsi with pale markings less distinct than on front legs. Hind tarsi with a few pale scales at apices of first two segments forming, at most, very narrow rings ; last three segments without definite rings and usually entirely dark.

Wings (fig. 1). Pale scales creamy or slightly yellowish. Costa with the usual four pale spots rather small, especially the apical and subapical ; base of costa very variable, uninterrupted in one specimen, but usually with one or two pale interruptions which may be minute or of large size. First vein as costa, but with pale spot, variable in location, in the third main dark area ; accessory sector spot present ; basal portion, as usual, pale. Fourth vein and stem of second extremely variable, sometimes mainly dark with small pale spots, sometimes pale with dark spots ; branches of second vein mainly dark, upper branch with or without pale spot at middle. Fifth vein with small dark spot on stem at fork, dark spot near base of stem small, that at apex of lower fork of small to moderate size ; upper fork very variable. Sixth vein variable, outer half entirely dark or with a small or large pale spot. Fringe with dark spot, which may be extensive, at apex of wing ; distinct pale spot usually absent at apex of sixth vein ; indistinct pale spot sometimes present on basal portion of fringe. Outstanding scales on the whole of a rather narrow type.

Wing length. Well-grown bred ♀ :—about 4 mm.

ADULT. ♂. *Palps.* Club with the usual 3 pale spots or areas normally developed.

Terminalia. Parabasal spines as for group. Harpago with outer accessory hair delicate and from slightly more than half to 3/5 length of apical. Inner accessory hair absent, but microtrichia well-developed. Phallosome with about 6–10 pairs of leaflets, the longer ones of normal shape and serrated ; leaflets about 1/3–3/5 length of phallosome. (Two preparations examined.)

PUPA. *Paddle.* External border bare on basal half ; spines of fringe becoming gradually longer distally, and replaced by a few long hairs giving place to shorter ones which are not continued beyond the bristle. Apical bristle straight and relatively short, about 1/6–1/5 length of paddle. Accessory hair with about 3–5 branches and sometimes relatively large.

Spine A. VIII, normal ; VII, slender, finely pointed and about 1/3-2/5 length of segment ; VI and V, progressively shorter ; IV, very short.

Bristle B. VII, with about 2–4 branches ; VI and V, about 3–5.

Bristle C. VII, simple or bifid, longer than segment VIII ; VI and V, very delicate, much shorter than succeeding segments and with about 4–7 branches.

intervening dark band and involving the base of the fourth and apex of third segments ; proximal band very narrow, at apex of second segment.

Pharynx. Lateral flanges very small, about equal to those in *Neomyzomyia*. Only 7–9 pairs of well-developed rods and cones. Some, at least, of cones showing two rows of crest spines less widely separated than in *Neocellia* ; filaments narrow to moderately wide, serrated and sometimes split distally. Rods without well-marked bulbous bases ; filaments about equal to those of cones. Hairs of post-armature ridges mostly short, but one or two may be developed into supernumary filaments.

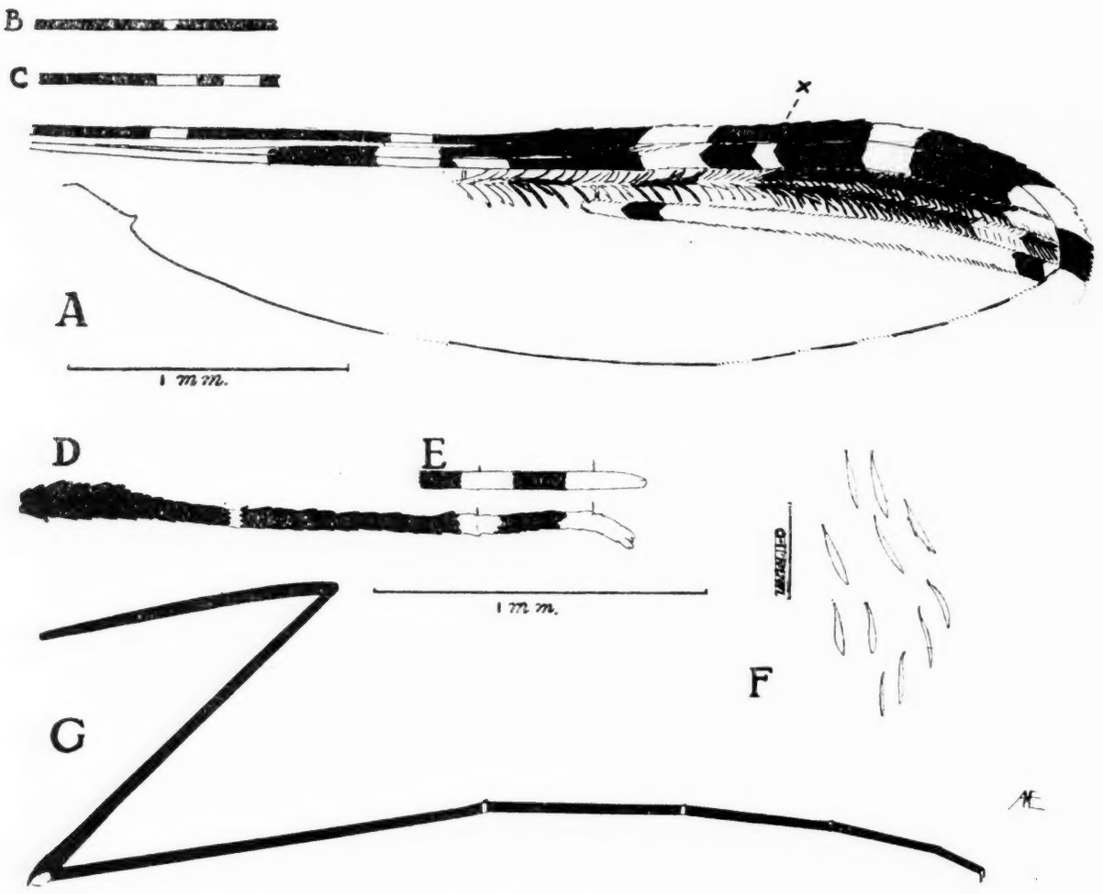


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Bristle C'. VI, simple to trifid.

Hair No. 4. VII, about 5 branches ; VI, 5–7 ; V, 6–8.

Respiratory trumpet. Meatus rather less than 1/3 length of trumpet.

LARVA. Clypeal hairs all simple. Inner mid frontal hairs very long. Pleural hairs as group *Myzomyia*, subgroup I, but both branched bristles relatively sparsely feathered. Leaflets of typical palmate hairs very variable

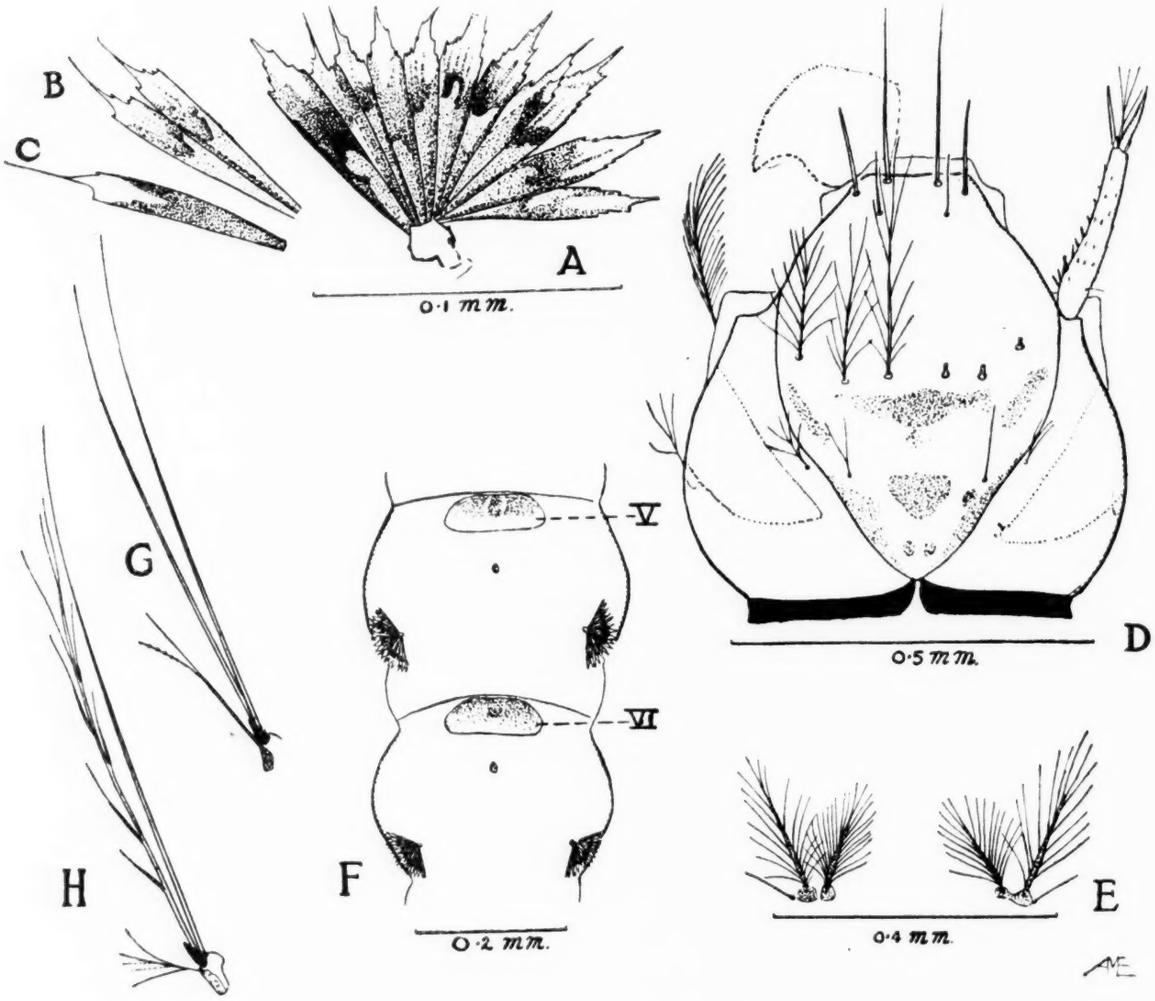


FIG. 2. *Anopheles harperi.* Larval details. A to C.—Leaflets from palmate hairs of fifth and fourth abdominal segments of different specimens, showing variation ; D.—Head, dorsal aspect (some details omitted) ; E.—Shoulder hairs ; F.—Fifth and sixth abdominal segments to show tergal plates ; G.—Mesopleural bristles ; H.—Metapleural bristles.

in shape. Main tergal plates much as in *demeilloni.* Lateral bristle on abdominal segment III, sparsely feathered. Saddle hair long and simple. Pecten of normal type.

A rather large larva resembling that of *A. natalensis* to the naked eye.

Head (fig. 2). Fronto-clypeus without dark markings on anterior half. Clypeal hairs simple : inner of normal length ; outer rather more or less than

half length of inner; posterior somewhat shorter than outer. Mid-frontal hairs sparsely branched, inner relatively very long, about half as long again as adjacent hairs. Post-frontal hairs simple or bifid. Antenna with spicules slender, a group of longer ones present towards base of inner border; shaft hair of moderate size and situated at about 1/4–2/7 from base, apical hair split into about 4 or 5 branches.

Thorax (fig. 2). Shoulder hairs with bases near together, either fused or separate; hairs with stems not flattened, inner with about 20–29 branches. Palmate hair with about 11–13 very narrow leaflets. Pleural hairs as in group *Myzomyia*, subgroup I (including *A. funestus*), but anterior dorsal with only about 8 or 9 branches on prothorax and about 7–9 or less on metathorax.

Abdomen (fig. 2). *Palmate hairs.* I, with about 11–14 very narrow leaflets, serrations absent or just indicated; II, with about 12–18 very narrow leaflets with definite serrations and filament; III—VII, well-developed, that on V with about 17–20 leaflets 0·08–0·18 mm. in length. Leaflets commonly rather broad, index about 4·5–6, with numerous serrations and gradually sloping shoulders, but sometimes narrow with few serrations and abrupt shoulders; filaments either very short and stumpy or longer, occasionally almost half length of blade, with well-marked linear terminations. Leaflets usually rather heavily pigmented at outer half. Saddle hair long and simple. Tergal plates of moderate size, as in *A. demeilloni*; paired accessory plates absent. Lateral hair on segment III sparsely feathered, only about 9–11 branches; on IV—VI bifid or trifid. Median plate of scoop without definite arms. *Pecten* with about 5 long teeth and 3 or 4 groups of short teeth, numbers in groups very variable.

Egg. Unknown.

Breeding Place. Numerous larvae were found in a backwater of a dam in a tributary of the 'Nzoia river. The water was shallow, with standing grass and shaded by trees and bushes; tall reeds separated the site from the rest of the dam, which harboured larvae of *A. funestus* type form.

Distribution. Kenya. Type ♂ and ♀ and paratype ♂♂ and ♀♀ with larval and pupal pelts, Kitale, farm 7 miles from the town in 'Nzoia river belt, collected by J. O. Harper, June 11th, 1936, and bred out by the writer. Three ♀♀ taken at Kericho in 1929 by the Kenya Medical Department are referable to this species.

This species is markedly distinct from all other African *Myzomyias*. The presence of a pale interruption in the third dark area on the first vein, together with absence of pale rings on the third and fourth tarsal segments, makes it easy to separate on colouration characters; most ♀♀ also have the subapical palpal band much shorter than in other *Myzomyias*, in which the first-mentioned character is present, except in some specimens of *A. hargreavesi*. The exceptional condition of the pharynx and of certain important larval bristles, referred to above, show that *A. harperi* is not at all closely related to any African species in which these characters are known.

The species is named in honour of its collector, Mr. J. O. Harper.

Type ♀ in the British Museum ; type ♂ in the Liverpool School of Tropical Medicine.

Anopheles (Myzomyia) macmahoni sp.nov.

LARVA. A rather small larva, with tergal plates resembling those of *A. longipalpis*, but one of the long mesopleural hairs feathered.

Size. About 5 mm. (Larvae mounted in lactophenol.)

Head (fig. 3, D). Anterior half of fronto-clypeus usually without dark markings, but sometimes with a pair of irregular brown areas. Inner clypeal hairs simple ; outer simple, about half length of inner ; posterior about equal to outer, simple or with end bifid or trifid. Mid-frontal hairs normal. Post-frontal hairs simple in all cases seen. Antenna with well-developed spicules,

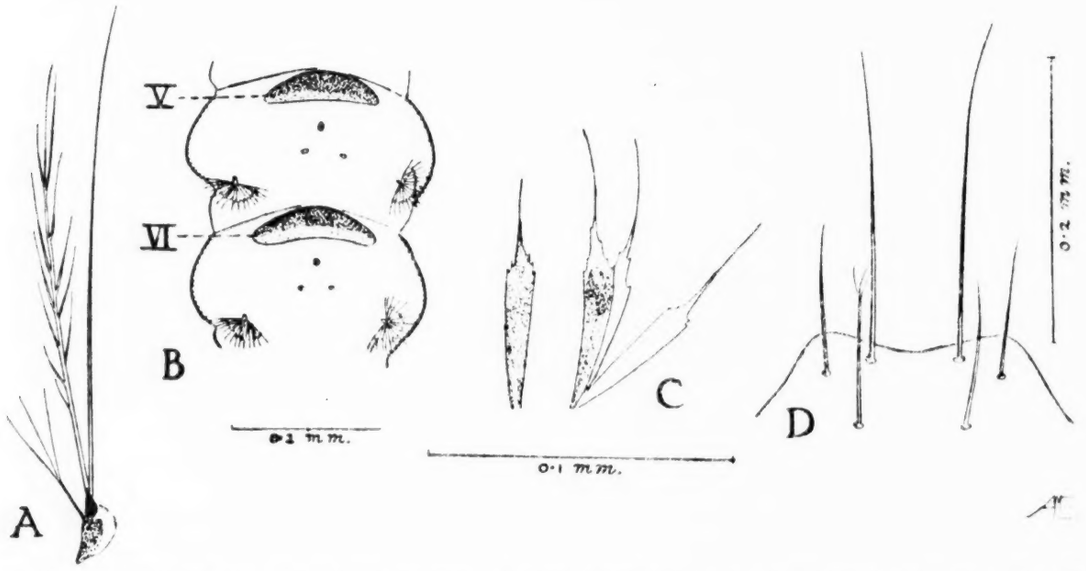


FIG. 3. *Anopheles macmahoni* sp.nov. Larval details. A.—Mesopleural bristles ; B.—Fifth and sixth abdominal segments to show tergal plates ; C.—Leaflets from palmate hair of fifth abdominal segment ; D.—Clypeal hairs.

not longer on basal 1/3 of internal border than elsewhere ; shaft hair of moderate size at basal 1/4 or more ; apical hair split into about 4–8 branches ; papilla distinctly longer than finger-like process.

Thorax (fig. 3, A). Shoulder hairs with chitinized bases, those of inner and middle hairs usually separate, but not widely so ; stems not flattened ; inner hair with about 12–17 branches. Palmate hair with about 10–13 very narrow leaflets. Pleural bristles as in group *Myzomyia*, subgroup II ; meso-pleural anterior dorsal with about 10–14 branches.

Abdomen (fig. 3, B, C). Palmate hairs. I, with about 11–13 very narrow leaflets, with just perceptible shoulders and long filaments ; II, small, but leaflets definitely shouldered ; III—V, rather small, that on V with about 16–19

leaflets less than 0·1 mm. in length. Blades rather narrow and filaments relatively long, usually about half to almost whole length of blade, with well-marked linear termination. Leaflets either uniformly pigmented or with dark area on outer half, Saddle hair simple. Main tergal plates unusually broad, very similar to those of *A. longipalpis* ; on V at least 3/4 distance between palmate hairs, and on VI almost equal to the distance. The plates may be deeper than as shown in fig. 3, B, and on VI and VII, and sometimes on other segments, the median accessory tergal plate may project from the margin of the main plate. Paired accessory plates (sometimes very small) usually present on most of abdominal segments IV—VI. Laterals on IV—VI with about 4–6 branches. Median plate of scoop with well-developed arms much as in *A. funestus* type form. Pecten with 4 or 5 long teeth and with 3 or 4 groups of short teeth, which are usually about 1/2–2/3 the length of the ventral long tooth ; ventral group usually with about 3 or 4 teeth ; spicules well developed.

Pupa. The pupa is very similar to that of *A. demeilloni.*

Paddle. Outer half with fringe of fine spines merging gradually into hairs which are not continued past the bristle. Apical bristle long and hooked. Accessory hair branched (6 in 1 specimen).

Spine A. VIII, with normal branching ; VII, slender and rather more than half length of segment ; VI and V, progressively shorter ; IV, much shorter than that on V.

Bristle B. VII—V, with about 3–5 branches.

Bristle C. VII—V simple, distinctly longer than segments on V and VI.

Respiratory trumpet. Meatus about 1/4 length of trumpet.

Adults. The associated adults bear a strikingly close resemblance externally to *A. funestus* type form. ♀ palpi with 3 narrow pale bands. Tarsi without definite pale rings. Wings predominantly dark ; third vein mainly or entirely dark.

Thorax. The mesonotum is rubbed in most of the specimens, but in one specimen the scales are whitish and mainly very narrow, with a few somewhat broader ones intermixed.

Legs. Hind tibiae mostly with a rather distinct white spot at base, as well as the usual apical spot.

Wings. Pale scales white. Costa with basal pale interruption present in all specimens and well developed in three. Third vein entirely dark or with a pale spot not more than 1/3 its length situated distally, as in *funestus.*

Wing length. 3·0–3·2 mm. (bred ♂♂ and ♀♀).

Pharynx. The pharynx of one ♀ was examined. Lateral flanges of moderate size. Fifteen pairs of rods and cones, the rods with well-marked bulbous bases. The row of post-armature ridges adjacent to the rods appear to have the central process developed into a very narrow filament. The writer cannot, however, be quite certain of this point from the single mount.

♂. *Palp.* Club in one specimen with three pale areas, the pale spot at

the base quite distinct. In the other specimen the apical pale area is present, and the pale spot at the base is indicated but is not very distinct.

Terminalia. Differing from that of *A. funestus* type form in the slightly longer external accessory harpago hair, which is a little more than half the length of the apical bristle.

The larvae of this species can be identified easily by the characters of the tergal plates in combination with those of the mesopleural bristles (fig. 3). They differ from those of *A. demeilloni* both in the former character and in the much smaller blades of the palmate hair leaflets and shorter posterior clypeals, as well as in other details, some of which tend to vary in *demeilloni*. The species also appears to be smaller than *demeilloni* and, were it not for the larval characters, the adult would have been considered to be a form of *A. funestus*.

DISTRIBUTION. So far only known from Isiolo, Kenya, the material all collected or reared by the Department of Medical Entomology. Cotypes, 2 larvae taken during or before 1935. Other material, 1 ♂ and 1 ♀ with pelts, June-July, 1936; 1 ♂ and 1 ♀ from same series; several larvae.

Cotypes in the Liverpool School of Tropical Medicine.

The species is named in honour of Mr. J. Macmahon, who has devoted much time to a study of the larvae.

THE DISSOCIATION OF ANTIBODY FROM SENSITIZED TRYPANOSOMES*

BY

E. M. LOURIE

(*Beit Memorial Research Fellow*)

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In a recent study of trypanolysis *in vitro* (Lourie and O'Connor, 1936) experiments were described in which pathogenic trypanosomes were lysed by mouse immune serum within half an hour at 37° C., in the presence of haemolytic complement; when trypanosomes had been sensitized by contact with immune serum for 1 hour at 0° C., and then washed at room-temperature, lysis similarly took place in half an hour at 37° C. in the presence of complement. It was shown that trypanolysis could be effected by mouse immune serum even in the complete absence of complement, the process then taking place in about 10–18 hours at 37° C.; but there was no lysis of washed sensitized trypanosomes even after 24 hours at 37° C. in the absence of complement. These facts may be summarily stated thus :—

(*a*) Complement	+ immune serum + trypanosomes	——>	Lysis in ½ hour.
(*b*) „	+ washed sensitized trypanosomes	——>	„ „
(*c*) Heated guinea-pig serum	+ immune serum + trypanosomes	——>	Lysis in 10-18 hours.
(*d*) „ „ „	+ washed sensitized trypanosomes	——>	No lysis in 24 „

The explanation of the failure of sensitized trypanosomes to lyse within 24 hours in the absence of complement may be that sufficient antibody for this purpose is not absorbed by the organisms during the process of sensitization, or may not be retained during subsequent washing. Assuming, however, that when they are deposited in previously heated guinea-pig serum the sensitized trypanosomes are indeed furnished with sufficient antibody for lysis within 24 hours in the absence of complement, then the fact that this result does not arise may be explained simply by the dissociation or diffusion of antibody into the surrounding medium upon standing. The latter explanation is supported by the experiment described below, in which we have demonstrated the dissociation of antibody from sensitized trypanosomes; the experiment includes some observations upon the influence of temperature on the rate and extent of this dissociation.

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Experiment.—A large number of trypanosomes (*T. rhodesiense*) was obtained from a heavily infected mouse, washed and then brought into contact, at 0° C. for 1 hour, with about 1 c.cm. of mouse immune serum which had previously been heated to 56° C. for half an hour (titre of immune serum—256); during their contact with the immune serum the trypanosomes were present in a concentration of about 10,000 per c.mm. They were then rapidly washed three times at 0° C. in deactivated guinea-pig serum 1 part, Ringer-glucose solution 3 parts, in about 1 c.cm. of which medium they were finally suspended. There was then sown 1/40th c.cm. of this suspension of sensitized trypanosomes into a series of small tubes containing 0·45 c.cm. of guinea-pig serum deactivated by heating to 56° C. for half an hour. One set of these tubes was kept at 37° C. (see Table; tubes 1–7) and another was left at room-temperature (tubes 8–18). At varying intervals one tube was taken from each set, the contents centrifuged, and the deactivated guinea-pig serum removed from the trypanosomes and replaced by an equal volume of complement (fresh unheated guinea-pig serum). The mixture was then stirred and kept in a water-bath at 37° C. for half an hour, lysis being measured by counting the trypanosomes present before and after this period of incubation with complement.

It is clear that trypanolysis during the half-hour contact with complement must indicate that antibody had remained intimately associated with the sensitized trypanosomes, whilst the failure of lysis would imply that antibody must have ceased to be bound to the organisms at some time during their previous sojourn in the deactivated guinea-pig serum. From the results shown in the Table it is to be inferred, then, that dissociation of antibody from sensitized trypanosomes does indeed take place upon standing in deactivated serum, and that its extent and rate of progress are markedly influenced by temperature. Thus, at 37° C. the dissociation after about 1 hour (tube 5) was already the maximum demonstrable by the technique used; at room-temperature, however, this point was not reached after even 12 hours (tube 14), and no further progress was measured between the 12th and 24th hour (tubes 14–18). After 24 hours at room-temperature, therefore, the dissociation was still not as far advanced as after 1 hour at 37° C.

SUMMARY

Trypanosomes sensitized by contact with mouse immune serum at 0° C. for 1 hour were washed and allowed to stand, at 37° C. and at room-temperature respectively, in a number of tubes containing deactivated guinea-pig serum. At varying intervals one of these tubes was taken, the deactivated serum replaced by complement, and the tube kept at 37° C. for half an hour. Lysis during this contact with complement indicates that antibody had remained bound to the trypanosomes, while absence of lysis implies a dissociation of antibody from the sensitized organisms during their previous sojourn in deactivated serum. In

TABLE

Showing the dissociation of antibody from washed sensitized trypanosomes upon standing in deactivated guinea-pig serum at 37° C. and at room-temperature, respectively. Dissociation indicated by failure of trypanosomes to lyse, on replacing the deactivated serum by complement

Tube	Temperature at which sensitized trypanosomes remained in contact with deactivated serum	Duration of contact of sensitized trypanosomes with deactivated serum	No. of trypanosomes per 256 squares of haemocytometer scale immediately after replacing deactivated serum by complement	No. of trypanosomes per 256 squares of haemocytometer scale after contact with complement for half an hour at 37° C.	Percentage of trypanosomes lysed during contact with complement
1	37° C.	5 minutes	34	0	100
2		¼ hour	21	0	100
3		½ „	29	16	45
4		¾ „	27	23	15
5		1 „	30	32	0
6		1¼ hours	24	25	0
7		1½ „	29	32	0
8	Room-temperature (17-19° C.)	2 hours	32	0	100
9		3 „	26	0	100
10		4 „	30	1	97
11		6 „	26	8	69
12		8 „	23	13	43
13		10 „	25	15	40
14		12 „	21	17	19
15		14 „	32	21	34
16		16 „	24	18	25
17		20 „	33	20	39
18		24 „	19	15	21

this way the dissociation of antibody from sensitized trypanosomes has been demonstrated, and has been shown to proceed at a considerably greater pace at 37° C. than at room-temperature (17–19° C.). Even after 24 hours at room-temperature the dissociation was much less advanced than after 1 hour at 37° C.

REFERENCE

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